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# Sleep-related activity and recovery of function in the somatosensory cortex during early development

Amy Jo Marcano-Reik  
*University of Iowa*

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**SLEEP-RELATED ACTIVITY AND RECOVERY OF FUNCTION IN THE  
SOMATOSENSORY CORTEX DURING EARLY DEVELOPMENT**

by

Amy Jo Marcano-Reik

An Abstract

Of a thesis submitted in partial fulfillment of the requirements for the  
Interdisciplinary Studies-Ph.D. degree in Neural Systems and Development in  
the Graduate College of The University of Iowa

December 2011

Thesis Supervisor: Professor Richard Shields

## ABSTRACT

The corpus callosum, the largest and major interhemispheric fiber tract, mediates communication between homotopic regions within the primary somatosensory cortex (S1). Recently, in 1- to 6-day-old neonatal rats, brief bursts of high-frequency, oscillatory activity – called spindle-bursts (SBs) – were described in S1 following sensory feedback from endogenously generated sleep-related myoclonic twitch movements and exogenously generated peripheral stimulation. To determine whether interhemispheric communication via the corpus callosum modulates the expression of SBs during this early period of development, we investigated the endogenous (spontaneous) expression and exogenous (evoked) activity of SBs in neonatal rats with intact or surgically severed callosal fibers (i.e., callosotomy; CCx). Furthermore, S1 cortical development and plasticity associated with recovery of function after CCx. We used Ag/AgCl cortical surface electrodes in the S1-forelimb region of the cortex to measure neurophysiological and behavioral activity in both intact and CCx subjects across the sleep-wake cycle during the first two postnatal weeks of development.

Our results demonstrate, for the first time, that the corpus callosum modulates spontaneous and evoked activity between homotopic regions in S1 as early as 24-hours after birth. In addition, CCx disinhibits cortical activity, nearly doubling the rate of spontaneous SBs through, but not after, postnatal day 6 (P6). CCx also significantly and reliably disrupts the evoked response to peripheral stimulation of the forepaw. To examine the role of sleep-related twitches and their associated sensory feedback (SBs in S1) – modulated by the corpus

callosum – in cortical development and plasticity, we performed CCx or sham surgeries at P1, P6, or P8, and tested subjects the day of surgery or over the ensuing week of recovery. Regardless of age, CCx immediately disrupted SBs evoked by forepaw stimulation. The P1 and P6 CCx groups exhibited full recovery after one week; in contrast, the P8 group did not exhibit recovery of function, thus indicating an abrupt decrease in cortical plasticity between P6 and P8.

Together, these results provide the first evidence that sleep-related myoclonic twitches and the associated sensory feedback in S1 (SBs) contribute to cortical development, plasticity, and recovery of function after interhemispheric communication is disrupted by callosotomy. CCx-induced disinhibition of spontaneous SBs is a transient phenomenon whose disappearance coincides with the onset of increased intrinsic connectivity, establishment of excitatory-inhibitory balance, and diminished plasticity in S1. Our findings indicate that CCx-induced disinhibition of spontaneous twitch-related SBs and disruption of evoked response to peripheral stimulation serve as a bioassay of somatosensory cortical plasticity during the early postnatal period.

Abstract Approved:

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Thesis Supervisor

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Title and Department

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Date

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Graduate College  
The University of Iowa  
Iowa City, Iowa

CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

Amy Jo Marcano-Reik

has been approved by the Examining Committee for the thesis requirement for the Interdisciplinary Studies-Ph.D. degree in Neural Systems and Development at the December 2011 graduation.

Thesis Committee:

\_\_\_\_\_  
Richard Shields, Thesis Supervisor

\_\_\_\_\_  
Alan Kim Johnson

\_\_\_\_\_  
Amy Poremba

\_\_\_\_\_  
Warren Darling

\_\_\_\_\_  
Cynthia Kubu

To Bailey and Shelby



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## TABLE OF CONTENTS

LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vi
CHAPTER	
I. BACKGROUND AND INTRODUCTION	1
Cortical plasticity	1
Interhemispheric communication	3
II. SLEEP-RELATED MYOCLONIC TWITCHES INFLUENCE CENTRAL NERVOUS SYSTEM DEVELOPMENT	8
Materials and methods	10
Results	18
Discussion	31
III. AN ABRUPT DEVELOPMENTAL SHIFT IN CALLOSAL MODULATION OF SLEEP-RELATED SPINDLE BURSTS COINCIDES WITH THE EMERENCE OF EXCITATORY-INHIBITORY BALANCE AND A LOSS OF SOMATOSENSORY CORTICAL PLASTICITY: DISINHIBITION OF SLEEP-RELATED SPONTANEOUS SPINDLE BURSTS AS A BIOASSAY OF SOMATOSENSORY CORTICAL PLASTICITY	36
Materials and methods	37
Results	48
Discussion	63
IV. GENERAL DISCUSSION	71
REFERENCES	75

## LIST OF FIGURES

### Figure

1.	Spontaneous and evoked spindle bursts (SBs) in a P5 rat....	13
2.	Spontaneous spindle-bursts (SBs) in intact and callosotomized P1-6 rats.....	20
3.	Effects of bupivacaine and scopolamine on evoked SBs in P1-6 rats.....	24
4.	Callosotomy reinstates contralateral evoked spindle-bursts (SBs) after scopolamine infusion.....	27
5.	Dissociation of tactile and proprioceptive S1 responses in newborn rats.....	30
6.	Changes in SB activity in sham and callosotomized (CCx) P2-15 rats.....	49
7.	Developmental changes in percentage of time asleep and rates of active sleep-related twitching in sham and callosotomized (CCx) P2-15 rats.....	51
8.	Effect of unilateral infusion of ACSF, muscimol, or bicuculline into S1 on spontaneous SB activity in sham and CCx P4 rats.....	53
9.	Cortical KCC2 protein levels increase at the end of the first postnatal week.....	56
10.	Changes in GABAergic and glutamatergic presynaptic terminals in P6-9 rats.....	58
11.	Callosotomy (CCx) disrupts the SB response to peripheral stimulation of the contralateral forepaw in P2-15 rats.....	60
12.	Disruption of evoked spindle burst responses by callosotomy (CCx) and recovery of function across different ages.....	62
13.	Model depicting hypothesized mechanisms underlying the development of excitatory-inhibitory balance in S1 between P6 and P10.....	67

## LIST OF ABBREVIATIONS

ACSF: artificial cerebrospinal fluid  
AgCC: agenesis of the corpus callosum  
Ag/AgCl: silver/silver chloride  
AS: active sleep  
BDNF: brain-derived neurotrophic factor  
Ca<sup>2+</sup>: calcium  
CC: corpus callosum  
Cl: chloride  
CCx: callosotomy  
EEG: electroencephalogram  
EMG: electromyogram  
GABA: Gamma-aminobutyric acid  
GAD: glutamic acid decarboxylase  
KCC2: K<sup>+</sup>/Cl<sup>-</sup> co-transporter 2  
L<sup>-</sup>/L<sup>+</sup>: interhemispheric SB latency  
NREM: non-rapid eye movement  
P: postnatal day  
REM: rapid eye movement  
S1: primary somatosensory cortex  
SB: spindle burst  
V1: primary visual cortex  
VGLUT-1: vesicular glutamate transporter-1

## CHAPTER I BACKGROUND AND INTRODUCTION

### Cortical plasticity

Plasticity is defined as the ability of the nervous system to change in an experience-dependent manner. Nearly every level of the nervous system exhibits some form of plasticity including the spinal cord (Dobkin, 1998; Fawcett, 1998; de Leon et al., 1999; Rossignol, 2000; Raineteau & Schwab, 2001; Thompson, 2001; Wolpaw & Tennissen, 2001; Rosenzweig, 2010; Dietz, 2011; Onifer, 2011) and subcortical regions (Garraghty & Kaas, 1991; Skoe, 2010; Kealy, 2011; Monsey, 2011; Xerri, 2011). Reorganization of new connections (i.e., synapses) has the potential to occur post-injury in response to damage. As described by Hebbian theory, synaptic plasticity is the result of any change in synaptic efficacy or connectivity between two neurons and underlies plasticity necessary for learning and memory to occur (Hebb, 1949; Kolb, 1999; Morris, 1999).

Cortical plasticity is a dynamic process that is continuously modified by experience (Buonomano & Merzenich, 1998; Froemke, 2007; Butz, 2009; Feldman, 2009; Kerr, 2011; Pita-Almenar, 2011). This phenomenon is often examined in the form of cortical map establishment and restructuring after cortical damage, or loss of peripheral inputs, and can be induced by a number of factors including alterations at the behavioral level.

Merzenich and colleagues have demonstrated that cortical plasticity persists into adulthood (Merzenich et al., 1983a; Merzenich et al., 1983b; Merzenich et al., 1984; Wall et al., 1986). In monkeys, transections of the medial nerve, which control sensory and motor function in the hand, alter the representations of the digits in somatosensory cortex. After transection, the

cortical representations are reorganized and bordering cortical zones of other skin surfaces begin to invade the transection-induced non-innervated cortex, resulting in new cortical representations of the skin surface. Over time, the remaining intact nerves reorganize and occupy larger portions of cortex than the intact, original inputs had previously occupied. In fact, in a series of additional experiments, two to eight months post-transection, most of the cortex responded to the remaining, intact skin surfaces and no longer responded to the original, transected digits (Merzenich et al., 1984). Therefore, the cortical zones had reorganized to represent the new and intact incoming sensory inputs. In a similar study, when digits were surgically fused together, cortical mapping was reorganized after a few months such that the individual digit map boundaries disappeared, and a fused, double digit cortical receptive field was formed (Clark et al., 1988; Allard et al., 1991). Thus, cortical maps are dynamic constructs that are organized by behavior and experience throughout the lifespan (Buonomano & Merzenich, 1998).

Although cortical plasticity persists into adulthood, it is most prevalent during early development. In young animals, recovery after damage is complicated by many factors including the extent of damage and age of the individual when damage occurred. For example, cortical lesions in infant mammals at different ages result in recovery of function at some ages but long-term deficits at others (Kennard, 1940; 1942; Passingham, 1983; Kolb, 1987; Kolb, 1991; Finger, 1999). The research conducted by Bryan Kolb and colleagues has been among the first to demonstrate that, in infant rodents, age and extent of lesion together result in different outcomes behaviorally and anatomically (Kolb, 1987; Kolb & Elliott, 1987; Kolb & Tomie, 1988; Kolb et al., 1996; Kolb, 2000; Kolb

& Cioe, 2000; 2003; Kolb & Gibb, 2007). One explanation for different outcomes, and different levels of plasticity across the lifespan, is that many factors that play a critical role in cortical organization fluctuate during development (Kolb et al., 2010). It is one aim of this dissertation to examine factors that influence primary somatosensory cortical plasticity and recovery of function during the early postnatal period.

Sleep and sleep loss may also modify experience-dependent cortical plasticity in vivo. Sleep in early life may play a crucial role in brain development (Roffwarg et al., 1966; Frank et al., 2001). Through a series of experiments, Frank and colleagues found that during a critical period of brain development, occluding the vision to one eye (monocular deprivation) resulted in rapid remodeling of the visual cortex and inputs. Sleep, but not wake, enhanced the effects of monocular deprivation and facilitated cortical plasticity in primary visual cortex (V1). Therefore, it is possible that sleep-related motor activity and its associated sensory feedback influence cortical plasticity and network organization during early development.

### **Interhemispheric communication**

The corpus callosum, the largest fiber tract in the brain, consists of more than 190 million axons (Tomasch, 1954; Banich & Belder, 1995). There is some debate as to whether the corpus callosum exerts an excitatory or inhibitory influence on cortical activity. This issue remains to be resolved (Bloom & Hynd, 2005). The excitatory functional viewpoint suggests that the corpus callosum actively integrates transcallosal information. The argument for an excitatory role is supported by the effect of split-brain surgery on epileptic seizure activity. This

procedure prevents interhemispheric spread of excitatory inputs. The inhibitory functional viewpoint suggests that the corpus callosum suppresses the contralateral hemisphere to allow for efficient processing of transcallosal activity (Boroojerdi, 1996; Avanzino, 2007). The argument for an inhibitory role is supported by a condition known as mirror movements of the limbs, where an individual actively attempts to move one limb and the contralateral limb moves in the same direction as the intended limb (i.e., it “mirrors” the intended limb), as a consequence of callosal disruption (Li et al., 2007a). In addition, physiological evidence supports transcallosal inhibitory functioning. Increased stimulus intensity in one hemisphere induces callosally mediated inhibition of the contralateral hemisphere (Meyer, 1995). These findings suggest the potential for intracortical inhibitory mechanisms.

Over half a century ago the first split-brain surgeries were performed in patients to control intractable epilepsy (Van Wagenen & Herren, 1940; Gazzaniga, 2005). The rationale for performing this procedure was the observation of two patients where a callosal tumor had decreased seizure frequency. This procedure was further supported by two additional patients that experienced a callosal insult which stopped seizure activity altogether. However, postsurgical testing of the patients showed no evidence of improvements in perceptual or motor abilities (Springer & Deutsch, 1999). Therefore, it was concluded that these surgeries were not successful in preventing seizure activity and the procedure was abandoned. In fact, after the failed attempts, it was concluded that the corpus callosum served the sole purpose of preventing the hemispheres from collapsing on one another ((Van Wagenen & Herren, 1940); for a review see Gazzaniga, 2005; Mooshagian, 2008).



The next split-brain surgery in humans was not conducted until many years later (Bogen & Vogel, 1962). Once Bogen and Vogel became aware of Sperry's and others' findings, they argued that Van Wagenen and Herren were probably unsuccessful at transecting the entire corpus callosum during their initial attempts at the procedure, and believed that to be the reason why the seizure activity continued. When they set out to perform the procedure again, on a former paratrooper who suffered from life-threatening seizures, they made certain that the entire corpus callosum was severed. This was the very first split-brain surgery that was successful in treating intractable epileptic seizure activity.

In a series of experiments conducted by Roger Sperry and a graduate student at the California Institute of Technology, ocular and behavioral functioning were examined in cats to investigate the function of the corpus callosum (Myers, 1956). These researchers used eye patches to cover an individual eye during the task to control visual input and processing. In addition, they transected the optic chiasm to control the transmission of information from the eye and the optic nerve. The experimenters then taught the cats a discrimination task where they were trained to press a lever when a certain stimulus was presented, and to not press when another stimulus was presented. The cats learned the task and performed well, until the researchers trained the cats with one eye covered and transected the corpus callosum. They found that, after callosotomy, when the eye patch was removed and then placed over the other (contralateral) eye, the cats were no longer able to perform the task. By cutting the callosal fibers and the optic chiasm, the uncovered eye learned the task, but that information was not transferred to the contralateral hemisphere. In

fact, the cats had to be re-trained. This experiment unequivocally showed that the corpus callosum transfers information between the two hemispheres.

The field has come a long way since the first split-brain surgery and early callosal functioning procedures were performed over fifty years ago. Since then, split-brain procedures have served as ways to examine the role of the corpus callosum in mediating interhemispheric communication (Van Wagenen & Herren, 1940; Ettliger et al., 1972; 1974; Koralek & Killackey, 1990; Lassonde et al., 1991; Orihara et al., 1997; Zepeda et al., 1999; Richards et al., 2004; Gazzaniga, 2005; Ren et al., 2006; Ribeiro-Carvalho et al., 2006; Paul et al., 2007; Marcano-Reik & Blumberg, 2008). However, the corpus callosum's influence on cortical organization and plasticity during early development remains to be examined.

During the prenatal and postnatal period in rats, activity-dependent processes guide the development of interhemispheric connectivity by the corpus callosum (Wang et al., 2007; Tagawa et al., 2008). Developmentally, many of the callosal fibers have crossed midline by the day of birth in rats and continue to grow into their target cortical layers over the first postnatal week until topographic distribution is established (Wise & Jones, 1976; Akers & Killackey, 1978; Nicoletis et al., 1991; Innocenti & Price, 2005b). The callosal projections arise from neurons in five of the six main cortical layers, with the exception of the neuron-sparse layer I (Koester & O'Leary, 1994). Both subplate neurons and pioneering axons play a crucial role in the development of the callosal projections. Distinct populations of projection neurons and developmental cues are necessary for the callosal projection pathways to form in the cortex (O'Leary & Koester, 1993).

The corpus callosum has also been shown to modulate interhemispheric cortical activity during sleep in adult humans. Interhemispheric coherence of activity is decreased during active, or rapid eye movement (REM), and non-rapid eye movement (NREM) sleep in people with congenital agenesis of the corpus callosum and after callosotomy (Corsi-Cabrera et al., 1995; Corsi-Cabrera et al., 2006). This occurs from a spread of coherent activity throughout callosally mediated thalamo-cortical circuits. These reports indicate that the callosal fibers may function in concert with sleep-dependent mechanisms to influence cortical activity in both intact and callosotomized conditions.

It is possible that sleep-related activity, modulated by the corpus callosum, contributes to sensorimotor organization during early development. Sleep occupies approximately 30% of our lives and predominates during early infancy (Roffwarg et al., 1966; Jouvet-Mounier et al., 1970; Karlsson et al., 2005), and myoclonic twitches of the muscles, which occur during REM sleep, are especially high during this early developmental period. Therefore, the primary goal of this dissertation is to examine how sleep-related myoclonic twitches and their associated sensory feedback influence somatosensory cortical plasticity and recovery of function after sensory feedback is disrupted by alterations to the corpus callosum (i.e., transection of the corpus callosum; callosotomy) during early development. It is possible that sleep-related myoclonic twitches serve as a primary source of sensory input driving activity-dependent processes that contribute to topographic connectivity and somatosensory cortical development, similar to the role of retinal waves for visual cortical organization and development (Katz & Shatz, 1996; Wong, 1999).

## CHAPTER II

### SLEEP-RELATED MYOCLONIC TWITCHES INFLUENCE CENTRAL NERVOUS SYSTEM DEVELOPMENT

The corpus callosum, which in humans consists of more than 190 million axons (Tomasch, 1954; Banich & Belder, 1995), mediates interhemispheric communication between homotopic cortical areas. Callosal fibers project to specialized cortical zones comprising maps within somatosensory cortex (Wise & Jones, 1976; Akers & Killackey, 1978; Innocenti & Price, 2005a). It is not known how disruption of these projections alters activity in somatosensory cortical areas and the development of somatotopy. Indeed, very little is known about callosal functioning early in development. Therefore, the primary goal of this dissertation is to examine the role of sleep-related behavior and callosally mediated activity on cortical organization and recovery of function after injury during the early developmental period. In addition, a model for the establishment of balance in the nervous system after injury (i.e., callosotomy) is proposed.

Such basic developmental information might help to explain the relationship between congenital malformations of the corpus callosum (including agenesis; AgCC) and a number of neurological and psychiatric disorders, including epilepsy, schizophrenia, and autism (Paul et al., 2007). Moreover, developmental investigation may help to resolve outstanding questions concerning the excitatory and inhibitory influences of callosal projections on cortical activity (Cook, 1984; Denenberg et al., 1986; Koralek & Killackey, 1990; Reggia et al., 2001; Bloom & Hynd, 2005).

Recently, it was reported that brief bursts of spatially confined oscillatory activity—so called spindle-bursts (SBs)—occur in primary somatosensory cortex (S1) in 1-6-day-old (P1-6) rats (Khazipov et al., 2004). SBs occurred in topographic

fashion as limbs exhibited fine twitch movements during sleep or high-amplitude movements during wakefulness; topographically related SBs were also evoked by tactile stimulation applied to various parts of the body. Subsequently, SBs were detected in barrel and primary visual cortex in association with whisker activity (Minlebaev et al., 2007) and retinal waves (Hanganu et al., 2006; Hanganu et al., 2007), respectively. In visual cortex, SBs were modulated by the cholinergic basal forebrain acting on cortical muscarinic receptors (Hanganu et al., 2007). It has been suggested that these topographically organized SBs reflect a self-organizational process underlying sensorimotor development (Khazipov et al., 2004; Khazipov & Luhmann, 2006).

The presence of topographically organized events in S1 offers the opportunity to assess interhemispheric communication during the early postnatal period when callosal projections are undergoing rapid developmental change. Specifically, many commissural fibers have crossed the midline by the day of birth in rats and, during the first postnatal week, callosal fibers grow into maturing cortex and topographic distribution is established (Wise & Jones, 1976; Akers & Killackey, 1978; Innocenti & Price, 2005a).

Here we investigate the expression of SBs in unanesthetized 1-6-day-old rats. We hypothesized that surgical or pharmacological manipulations that would be expected to disrupt or alter callosal communication would also alter SB activity in one or both S1s. Consistent with this hypothesis, transecting the corpus callosum (i.e., callosotomy) doubled the number of spontaneous, active sleep-related SBs in both S1s by reducing the interval between successive SBs. We also found that unilateral infusion of the muscarinic receptor antagonist, scopolamine, into the left S1 inhibited contralateral SB responses and

disinhibited ipsilateral SB responses evoked by right forepaw stimulation; subsequent callosotomy reinstated contralateral SB responses. These results establish a functional inhibitory role for the corpus callosum in newborn rats and provide a foundation for further investigation of the development of callosal function, its contribution to somatotopy, and recovery of cortical function after early callosal damage.

### **Materials and methods**

All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of animals in research and were approved by the Institution Animal Care and Use Committee of the University of Iowa. All efforts were made to minimize the number of animals used. Daily observations were conducted to ensure that all subjects maintained a visible milk band, which reflects recent feeding and appropriate maternal care.

**Subjects.** A total of 48 P1-6 rats from 48 litters were used. Males and females were equally represented among the subjects. Litters were culled to 8 pups within 3 days of birth (day of birth = P0). Mothers and their litters were housed in standard laboratory cages (48 cm x 20 cm x 26 cm) in the animal colony at the University of Iowa. Food and water were available ad libitum and all animals were maintained on a 12-hour light-dark cycle with lights on at 0700 hours. All experiments took place during the lights-on period.

**Surgery.** Under isoflurane anesthesia, a scalp incision was made in the anterior-to-posterior direction to expose the skull. The membranes were stripped away and the uncalcified skull was cleaned and Vetbond (3M, St. Paul, Minnesota, United States) was applied to strengthen the surface. As described

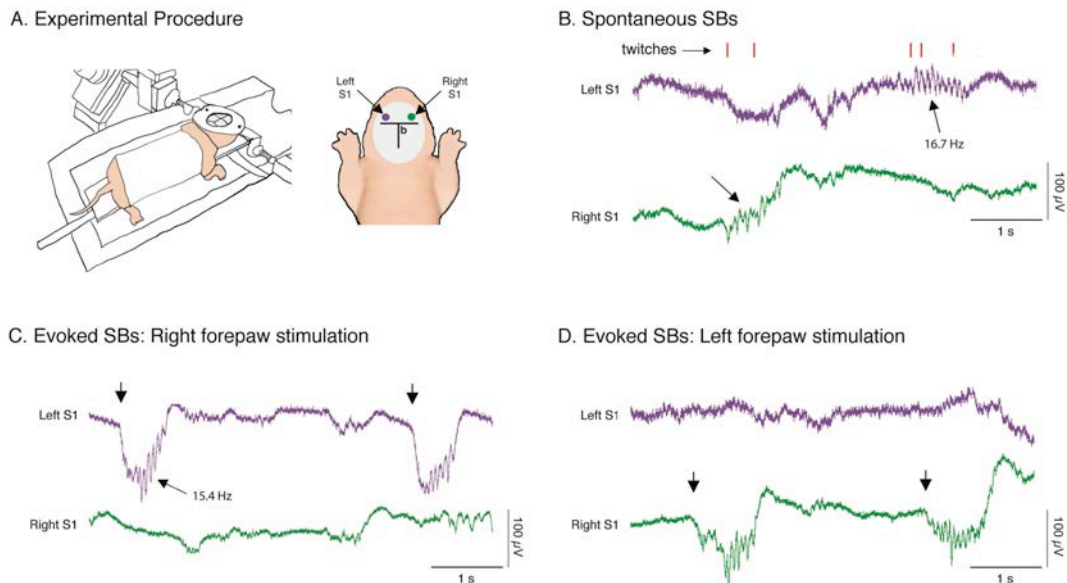
previously (Karlsson et al., 2005), a custom-built stainless steel apparatus, designed to attach to the earbar holders of a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, United States), was secured to the skull with cyanoacrylate adhesive. Bipolar stainless steel electrodes (50  $\mu\text{m}$  diameter, California Fine Wire, Grover Beach, CA) were inserted into the right nuchal and right biceps brachii muscles. A ground wire was implanted anterior to the nuchal EMG. All electrodes were secured with flexible collodion. At the end of surgery, the pup's trunk was lightly wrapped in gauze. These surgeries lasted approximately 10 min.

For those subjects that were callosotomized, the following procedure was added to that above: A 2-3 mm opening was created in the skull, halfway between bregma and lambda and parallel and just lateral to midline. A thin surgical knife was inserted to a depth of approximately 5 mm. The knife was then swept in an anterior and posterior direction to transect the corpus callosum primarily in its anterior half, which contains fibers that connect left and right S1s (Wise & Jones, 1976). Sham surgeries were identical except that the surgical knife was not inserted. This additional procedure added only 30 s to the surgery. In some subjects, the opening in the skull was created under anesthesia but the callosotomy was performed later during the recording session. We note that when the callosotomy was performed in these unanesthetized subjects, they never exhibited overt signs of distress; in fact, sleeping subjects typically remained asleep during the callosotomy.

Procedure. After surgery, each pup recovered for about 1 hour in a humidified incubator maintained at thermoneutrality (35°C), after which the pup was transferred to an electrically shielded chamber for testing. The pup's head

was fixed in the stereotaxic apparatus and its ventrum was placed on a flat support bar with its forelimbs and hindlimbs dangling freely on both sides without contacting any surface (see Figure 1A, left). Temperature-controlled water flowing through a concave double-walled glass chamber, situated beneath the pup, helped to control the thermal environment and, in conjunction with a heat lamp, maintain the pup's brain temperature at 37°C throughout testing.





**Figure 1. Spontaneous and evoked spindle bursts (SBs) in a P5 rat.** (A) Left: Experimental procedure for recording SBs. The infant rat was head-fixed in a stereotaxic apparatus, placed on a narrow platform, lightly wrapped in gauze, and suspended over a temperature-controlled glass chamber. A heating lamp was also used to maintain brain temperature at 37°C. Right: View of skull showing approximate location of electrodes in relation to bregma (b). Pairs of Ag/AgCl electrodes were placed in left (purple dot) and right (green dot) somatosensory cortex (S1) and SB responses to contralateral forepaw plantar surface stimulation were confirmed. (B) Spontaneous SBs (denoted by arrows) in left (purple) and right (green) S1 in relation to active sleep-related myoclonic twitches of the limbs (red ticks) assessed through behavioral observation. Contralateral SBs were produced in response to right (C) and left (D) forepaw plantar surface stimulation (denoted by arrows). Note that these evoked SBs, in contrast with the spontaneous SBs in (B), were embedded within large, slow potentials. The oscillation frequencies of one spontaneous and one evoked SB are also shown.

Two recording sites were prepared for placement of electrodes over the left and right S1s (see Figure 1A, right). Each site consisted of two holes separated by approximately 2 mm and centered over the forelimb region of S1 (Khazipov et al., 2004), approximately 1 mm rostral to bregma and 2-3 mm lateral to midline. Custom-made Ag/AgCl electrodes, consisting of Teflon-insulated silver wires (0.01 in. diameter; Medwire, Mount Vernon, NY) with approximately 1 mm of each tip stripped and chlorinated, were inserted just below the cortical surface. Ground electrodes were placed in the cerebellum.

Electroencephalographic (EEG) and electromyographic (EMG) electrodes were connected to differential amplifiers (A-M Systems, Carlsborg, WA; filter setting: 0.1-3000 Hz; amplification: x10,000). Neural and EMG signals were sampled at 12.5 kHz using a digital interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

After approximately 1 hour of acclimation, by which time sleep-wake cycles were evident, the plantar surface of the left and right forepaws was stimulated with a fine brush (typically, these strokes also elicited dorsiflexion of the wrist). Electrode placements were judged successful when plantar surface stimulation of the forepaw resulted in a corresponding SB embedded within a slow potential in the contralateral somatosensory cortex. When SBs were not detected, the electrode placements were adjusted. Importantly, other parts of the limb and body were stimulated to assure the specificity of the SB response.

EMG and EEG data were acquired continuously throughout the test. In all subjects, visual observation of sleep-wake behaviors and EMG recording of nuchal and right biceps brachii muscles provided measures of behavioral state (Karlsson et al., 2005). When appropriate, the experimenter scored sleep-related

myoclonic twitches as well as wake-related behaviors using a keyboard connected to the data acquisition system. As described elsewhere (Gramsbergen et al., 1970b; Karlsson et al., 2005), myoclonic twitches, indicative of active sleep, were defined as phasic, rapid, and independent movements of the limbs and tail. High-amplitude movements, indicative of wakefulness, included locomotion, stretching, and yawning. In addition, during periods when the plantar surface of the forepaw was stimulated, the experimenter simultaneously pressed a key on the keyboard to mark this event in synchrony with the electrophysiological data.

For the experiment in which subjects received callosotomy or sham surgery before testing, a 15-minute period in which myoclonic twitching and wake-related movements were scored was followed by a 15-minute period of forepaw stimulation. For the experiment in which subjects were callosotomized during testing, a 15-minute period of behavioral scoring during the pre-callosotomy period was followed by a 15-minute period of behavioral scoring during the post-callosotomy period (this latter period commenced with the completion of the callosotomy), followed by a final 15-minute period of forepaw stimulation.

For the experiment in which subjects were infused with artificial cerebral spinal fluid (ACSF), bupivacaine, or scopolamine, 15 minutes of behavioral scoring during the pre-infusion period was followed by 15 minutes of behavioral scoring during the post-infusion period (this latter period commenced with the completion of the drug infusion and sufficient time for drug diffusion; approximately 3-5 minutes). After the post-infusion period was complete, data were recorded during a final 15-minute period of forepaw stimulation. ACSF, bupivacaine hydrochloride (Abbott Laboratories, North Chicago, IL; 0.75%), and

scopolamine hydrochloride (Sigma-Aldrich, St. Louis, MO; 90  $\mu\text{g}/\mu\text{l}$  mixed in ACSF) were infused (0.1  $\mu\text{l}/\text{s}$ ) in a volume of 1  $\mu\text{l}$  using a Hamilton microlitre syringe with a 25-gauge needle (Model 7001, Hamilton, Reno, Nevada, United States) mounted directly above the infusion site. The syringe was lowered just below the cortical surface into a pre-drilled hole located midway between the 2 Ag/AgCl electrode sites. In a third experiment, three 15-minute periods of behavioral scoring and forepaw stimulations were recorded during (i) baseline, (Rivera et al.) after scopolamine infusion, and (iii) after callosotomy. Finally, in several subjects, scopolamine was again infused at the end of the experiment to assure that the observed effects of callosotomy were not due to a decrease in scopolamine's effectiveness over time.

A final experiment was conducted to examine the specificity of S1 responses to tactile and proprioceptive stimulation. For this experiment, surgical and recording procedures were the same as described above, but now several different kinds of forepaw stimulation, each occurring during successive 15-minute periods, were applied to all subjects while cortical activity was recorded. At least twenty stimulations in each category were presented to each subject and the ordering of the stimulation periods was the same in all subjects. Half of the subjects received left forepaw stimulation and the other half received right forepaw stimulation, with the order/group condition counter-balanced across subjects. First, using a fine brush, the plantar surface of the forepaw was stroked as in earlier experiments to produce tactile stimulation with wrist dorsiflexion; second, wrist dorsiflexion without plantar surface stimulation was produced by gently pulling a string attached to the dorsal surface of the paw (the string was attached earlier during surgery using cyanoacrylate adhesive); third, tactile

stimulation of the plantar surface of the forepaw was produced while being careful to avoid wrist dorsiflexion; fourth, as a further confirmation of the specificity of the proprioceptive response, a string was attached to the elbow joint with cyanoacrylate adhesive and the elbow was pulled repeatedly to a flexed position. Finally, tactile stimulation with wrist dorsiflexion was repeated to ensure that the recording conditions had remained stable throughout the period of testing.

**Histology.** Upon completion of testing, callosotomized pups were overdosed with an intraperitoneal injection of sodium pentobarbital and perfused transcardially with phosphate-buffered saline, followed by a 3% formalin solution. Brains were postfixed for at least 48 hours in a sucrose solution before being sliced in the coronal plane (50  $\mu\text{m}$  sections). Light microscopy was then used to assess the extent of damage to the corpus callosum.

**Data Analysis.** For each subject, 15-minute periods of synchronized data comprising digital records of behavior, EMG activity, and EEG activity were created and analyzed using Spike2 software. For all analyses, data were averaged for each subject before statistical analysis and paired (within-subjects) or unpaired (between-subjects) t tests were performed using JMP 5.0 software (SAS, Cary, North Carolina, USA). For all tests, alpha was set at 0.05.

Spontaneous and evoked SBs were identified by referring to both the raw EEG records of the left and right S1s as well as filtered records (band-pass: 5-40 Hz). Using criteria identical to those described previously (Khazipov et al., 2004), SBs comprised at least 3 complete oscillations, were  $> 100$  ms in duration, and contained at least one oscillation that exceeded 50  $\mu\text{V}$  in amplitude (from baseline to peak). In addition, evoked SBs were embedded in large, slow

potentials with amplitudes  $\geq 100 \mu\text{V}$ .

SB durations and latencies were compared between intact and callosotomized subjects. For this analysis, 20 “anchor” SBs in the left S1 recording were selected at random for each subject and its duration determined. Then, for each of these SBs, the latency between it and the prior (-L) and subsequent (+L) SBs in the right S1 recording was determined.

The effects of callosotomy and drug infusion on SB oscillation frequency were also analyzed. The frequency (in Hz) of an individual SB was determined by measuring the time between two successive oscillation peaks, averaging them, and then calculating the reciprocal. Mean SB frequency was determined for each subject by averaging across 20 individual SBs. Paired or unpaired t tests were then performed.

For each type of sensory stimulation presented to each subject in the final experiment (i.e., wrist flexion plus tactile stimulation, wrist flexion only, and tactile stimulation only), we determined the percentage of contralateral S1 responses that comprised SBs alone, slow potentials alone, or SBs embedded within slow potentials. The response data were imported into Statview 5.0 (SAS Institute, Cary, NC) and paired t tests were used to test for response differences within each stimulation category. Because multiple t tests were performed, a conservative alpha of 0.001 was used.

## **Results**

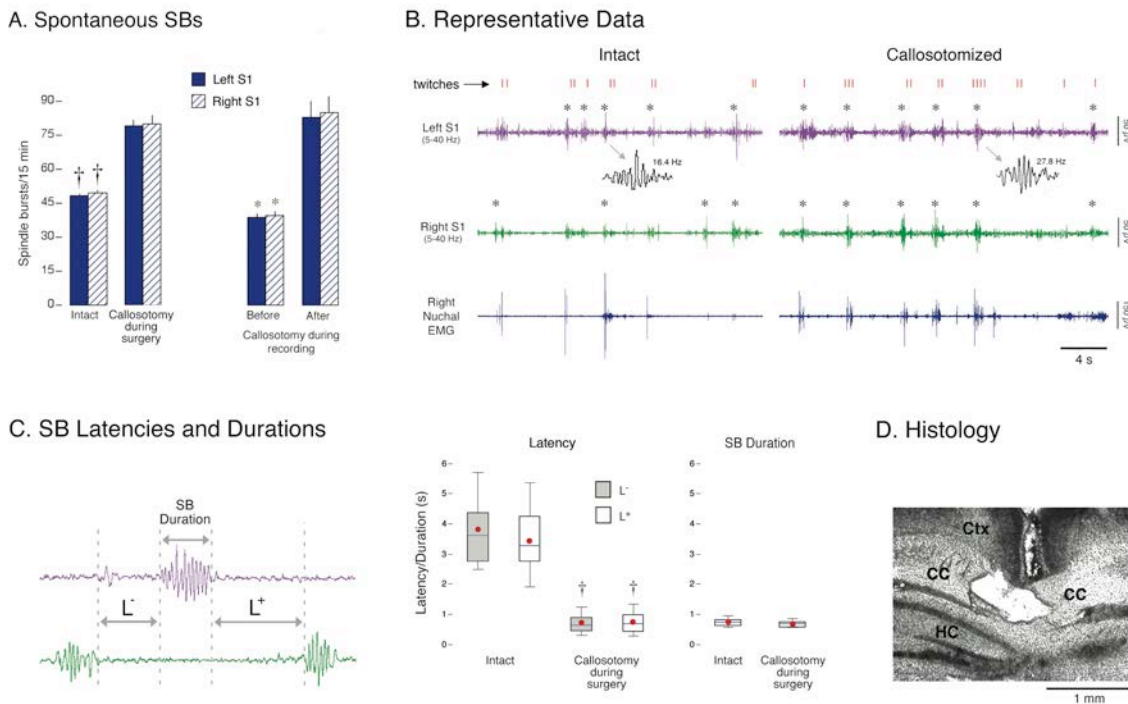
### Spontaneous and evoked S1 activity

In all experiments, cortical activity was examined in unanesthetized newborn rats, head-fixed and suspended in a prone position (Figure 1A).

Spontaneous SBs associated with sleep-related twitching of the distal limbs were reliably recorded (Figure 1B), as described previously (Khazipov et al., 2004). Also, stimulation of the plantar surface of each forepaw using a fine brush reliably evoked SBs in the contralateral S1 (i.e., > 95% of stimulations; Figure 1C, D). Under these experimental conditions, evoked SBs were distinguishable from those produced spontaneously by their being embedded within large, slow potentials with a duration typically exceeding 500 ms. Before further testing, we confirmed that SBs were specifically evoked by plantar surface stimulation of the forepaw by stimulating other parts of the body surface.

#### Effect of CCx on spontaneous and evoked SBs

Callosotomy resulted in a significant increase in spontaneous SBs in both left and right S1s (Figure 2A, left;  $t_{10s} > 7.6$ ,  $P_s < 0.0001$ ). Additionally, callosotomy resulted in a 60% increase in spontaneous SB oscillation frequency. Specifically, for intact subjects, mean SB frequency was  $17.1 \pm 0.3$  Hz, compared with  $28.9 \pm 0.6$  Hz for the callosotomized subjects ( $t_{10} = 18.2$ ,  $P < 0.0001$ ). In a second group of rats ( $n = 6$ ), callosotomies were performed during the recording session. In these animals, callosotomy produced an immediate increase in the rate of occurrence of spontaneous SBs in both S1s in relation to the pre-callosotomy period (Figure 2A, right;  $t_{5s} > 7.4$ ,  $P_s < 0.0001$ ); mean SB frequency also increased immediately and significantly after callosotomy (e.g., left S1, pre:  $17.3 \pm 0.4$  Hz; left S1, post:  $28.3 \pm 0.6$  Hz;  $t_5 = 17.0$ ,  $P < 0.0001$ ).



**Figure 2. Spontaneous spindle-bursts (SBs) in intact and callosotomized P1-6 rats.** (A) Left: Mean number of spontaneous SBs in left (solid) and right (hatched) S1 during 15-min recording periods. Two procedures were used: pups experienced sham surgery (intact) or callosotomy before recording ( $n = 6$  per group), or pups were callosotomized during the recording session ( $n = 6$ ). \*  $P < 0.001$  in relation to the callosotomy group or post-callosotomy period. Mean + s.e. (B) Representative periods of active sleep in an intact P6 subject and a callosotomized P5 subject. Traces depict right nuchal EMG and right (green) and left (purple) S1 EEG activity (band-pass filter: 5-40 Hz). Behaviorally scored myoclonic twitches of the limbs (red ticks) are also shown and confirmed SBs are denoted by asterisks. Note that SBs in both subjects occur reliably during periods of twitching. Two SBs are expanded to illustrate increased oscillation frequency after callosotomy. S1 activity sometimes increased during periods of twitching but was not categorized as an SB. This is because, upon closer examination, this activity did not exhibit the regular, sinusoidal features characteristic of SBs; instead, this non-SB activity was highly irregular in both frequency and amplitude. (C) Left: Illustration of the method used to measure SB durations and the latencies between SBs in different hemispheres. Right: Box plots depicting distributions of SB latencies and durations for intact and callosotomized subjects ( $n = 6$  per group). The top, middle, and bottom horizontal lines of the box represent the 75th, 50th (median), and 25th percentiles, respectively. The thin vertical lines above and below the box represent the 90th and 10th percentiles, respectively. Red circles are means. †  $P < 0.0001$  in relation to the intact group. (D) Coronal section showing severed corpus callosum (CC) ventral to cerebral cortex (Ctx) and dorsal to hippocampus (HC).



The callosotomy-induced increase in the occurrence of spontaneous SBs could have resulted from generalized functional disinhibition of these cortical oscillations, perhaps allowing them to occur independent of behavioral state. However, even in callosotomized pups, the close association between periods of myoclonic twitching and SBs was retained (Figure 2B). When we examined the number of limb twitches for each subject during the 15-minute recording period, we found no significant difference between the intact and callosotomized subjects (intact:  $343.8 \pm 25.1$  twitches; callosotomized:  $381.0 \pm 32.9$  twitches;  $t_{10} = 0.9$ ; similar results were found for subjects that were callosotomized during recording). This result was expected because, as reported previously in infant rats (Kreider & Blumberg, 2000), even complete transection of the brainstem between the cortex and mesopontine region does not alter the quantity or temporal organization of twitching.

Thus, we hypothesized that callosotomy exerted its effects on SBs by reducing mutually inhibitory interactions between left and right S1s. We tested this hypothesis by examining the temporal relationship between SBs in the left S1 in relation to SBs in the right S1 (Figure 2C, left). First, the nearly 4-second mean delay between interhemispheric SBs in the intact subjects is consistent with our observation that SBs in the two hemispheres almost never overlapped. Second, callosotomy produced a significant reduction in the latencies of interhemispheric SBs (Figure 2C, right;  $t_{10s} > 21.0$ ,  $P_s < 0.0001$ ). SB durations did not differ significantly between intact and callosotomized subjects ( $t_{10} = 0.3$ , NS). These findings suggest that callosotomy reduces interhemispheric inhibition and

thereby increases the probability that an SB will occur in response to twitch-related sensory feedback.

We might have expected SBs in the two disconnected hemispheres of callosotomized subjects to occasionally overlap if and when myoclonic twitches occurred simultaneously in left and right forelimbs. However, overlapping of SBs rarely occurred, which is consistent with the prior finding that myoclonic twitches are expressed in bouts comprising non-simultaneous movements of individual limbs (Robinson et al., 2000).

Finally, in callosotomized subjects, forepaw stimulation continued to evoke SB responses in the contralateral hemisphere, albeit approximately 30% less often than in intact subjects (e.g., left S1, intact:  $99.3 \pm 0.5\%$ ; callosotomy:  $69.4 \pm 6.7\%$ ;  $t_{10} = 4.4$ ,  $P < 0.005$ ). Also, as with spontaneous SBs, mean SB oscillation frequency in response to forepaw stimulation increased significantly in callosotomized subjects in relation to intact subjects (intact:  $18.1 \pm 0.4$  Hz; callosotomy:  $31.5 \pm 0.9$  Hz;  $t_{10} = 13.6$ ,  $P < 0.0001$ ). Again, similar results were found for subjects that were callosotomized during the test period.

#### Evoked SBs after scopolamine infusion

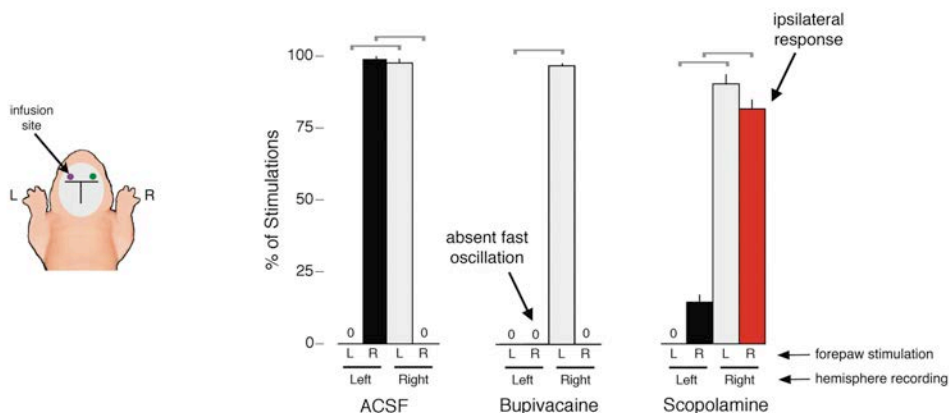
Previous studies have shown that network oscillations in newborn mouse neocortical slices are  $\text{Na}^+$ -channel dependent (Dupont et al., 2006) and that SBs in newborn rats are modulated by cholinergic input from the basal forebrain (Hanganu et al., 2007). Therefore, to alter S1 activity unilaterally so as to observe possible callosally mediated changes in the contralateral hemisphere, we examined the effects of the local anesthetic, bupivacaine, and the muscarinic receptor antagonist, scopolamine, on spontaneous and evoked SBs.

Bupivacaine's anesthetic properties have been attributed to its ability to block voltage-gated Na<sup>+</sup> channels as well as G protein-gated inwardly rectifying K<sup>+</sup> channels (Zhou et al., 2001; Kindler & Yost, 2005). The general procedure was to infuse artificial cerebrospinal fluid (ACSF; n = 4), bupivacaine (n = 6), or scopolamine (n = 6), just beneath the cortical surface in the left S1 (n = 6 per group), using methods similar to those described elsewhere (Hanganu et al., 2007). After infusion, spontaneous cortical activity was recorded and, thereafter, the plantar surface of the forepaws was stimulated.

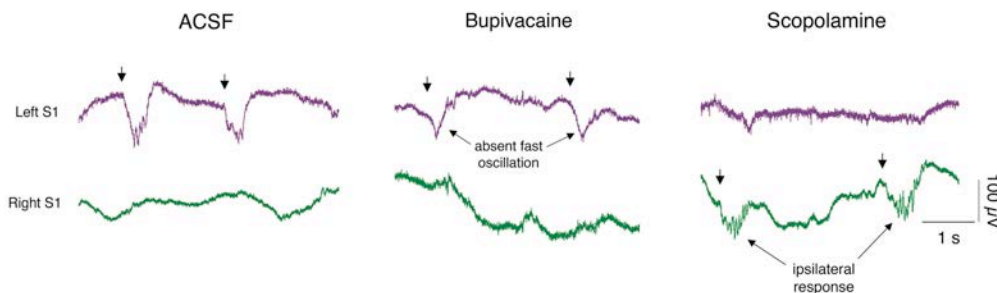
With regard to spontaneous SBs, neither bupivacaine nor scopolamine infusion into the left S1 significantly affected their rate of occurrence in either hemisphere in relation to the pre-infusion period ( $t_{5s} < 1.0$ , NS). For example, in the left hemisphere, the average spontaneous rate of occurrence of SBs before infusion of ACSF, bupivacaine, or scopolamine, respectively, was  $41.2 \pm 1.6$ ,  $49.3 \pm 1.4$ , and  $48.8 \pm 2.0$  SBs/15 minutes; after infusion, the respective values were  $39.75 \pm 1.7$ ,  $49.8 \pm 1.2$ , and  $48.0 \pm 2.6$  SBs/15 minutes.

With ACSF infusion into the left S1, SB responses in the left and right S1s occurred exclusively in response to contralateral forepaw stimulation (Figure 3A;  $t_{5s} > 72.5$ ,  $P_s < 0.0002$ ). For example, in the left S1, SBs occurred  $98.4 \pm 1.0\%$  of the time in response to right forepaw stimulation and never in response to left forepaw stimulation. In contrast, bupivacaine infusion eliminated SBs in the left S1 to right forepaw stimulation; however, contrary to our prediction, this elimination of SBs in the left S1 was not accompanied by an effect on right S1 activity. Moreover, although SBs were eliminated by bupivacaine, the large, slow potential was still reliably evoked, thus indicating that these two components are dissociable.

## A. Evoked SBs



## B. Evoked SBs: Right forepaw stimulation



**Figure 3. Effects of bupivacaine and scopolamine on evoked SBs in P1-6 rats.** (A) Mean percentage of evoked SBs in left and right S1s in response to stimulation of the plantar surface of the left (L) and right (R) forepaws. Each recording period was 15 min in duration. Pups were infused with artificial cerebrospinal fluid (ACSF;  $n = 4$ ), the local anesthetic, bupivacaine ( $n = 6$ ), or the muscarinic receptor antagonist, scopolamine ( $n = 6$ ), into the left S1. Brackets denote significant ( $P < 0.0001$ ) differences between hemispheres in response to left or right forepaw stimulation. Mean + s.e. (B) Representative evoked SBs in a P4 subject in left and right S1s in response to right forepaw stimulation (denoted by arrows). ACSF infusion did not affect the normal expression of contralateral SBs in the left S1. In contrast, scopolamine infusion resulted in the expression of ipsilateral SBs in the right S1. Finally, bupivacaine eliminated SB responding in the left S1 to right forepaw stimulation, although expression of the large, slow potential was unaffected.

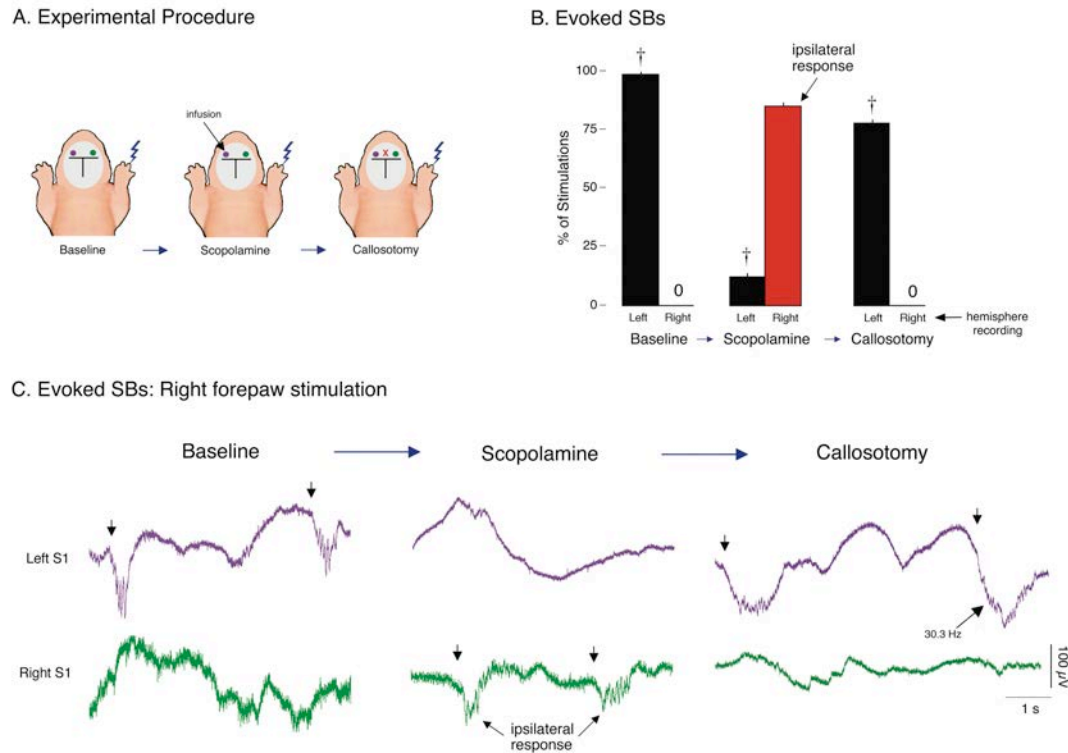
Scopolamine infusion into the left S1 did not interfere with the ability of left forepaw stimulation to evoke a contralateral SB response in the right S1 (contralateral:  $90.3 \pm 3.1\%$ ; ipsilateral:  $0.0 \pm 0.0\%$ ;  $t_5 = 29.2$ ,  $P < 0.0001$ ). However, stimulation of the right forepaw now significantly reduced contralateral SBs in left S1 and “unmasked” ipsilateral SBs in right S1 (contralateral:  $14.4 \pm 2.5\%$ ; ipsilateral:  $81.7 \pm 3.4\%$ ;  $t_5 = 11.6$ ,  $P < 0.0001$ ).

After infusion of ACSF into the left S1, mean SB oscillation frequency in the left S1 to right forepaw stimulation was  $17.2 \pm 0.2$  Hz, compared with  $17.8 \pm 0.5$  Hz in the right S1 in response to left forepaw stimulation. After infusion of scopolamine into the left S1, the ipsilateral SBs in the right S1 (non-drug) to right forepaw stimulation had a mean frequency of  $17.5 \pm 0.3$  Hz, which is not significantly different from the mean frequency of SBs produced in the right (non-drug) S1 to left forepaw stimulation ( $18.1 \pm 0.3$  Hz;  $t_5 = 1.1$ ); nor is this value significantly different from either ACSF group ( $t_{8s} < 0.8$ ). Therefore, in contrast to the effects of callosotomy, scopolamine infusion did not affect SB oscillation frequency.

#### Callosotomy reinstates evoked SBs

We next determined whether the ipsilateral expression of SBs after scopolamine infusion was mediated by the corpus callosum. Using 6 additional P3-6 rats, stimulations were applied to the right forepaw during 3 successive 15-minute periods: (i) during baseline, (Rivera et al.), ii) after scopolamine infusion into the left S1, and (iii) after subsequent callosotomy (Figure 4A). As expected, evoked SBs during the baseline period were expressed in the contralateral (i.e., left) S1 in response to right forepaw stimulation (Figure 4B;  $t_5 = 169.1$ ,  $P < 0.0001$ ).

Next, scopolamine infusion produced the expected ipsilateral SB response (Figure 4B;  $t_5 = 25.2$ ,  $P < 0.0001$ ). After callosotomy, ipsilateral SBs in response to right forepaw stimulations disappeared immediately and, to our surprise, contralateral responding was reinstated ( $t_5 = 53.2$ ,  $P < 0.0001$ ; representative data are presented in Figure 4C). Finally, to confirm that the reinstatement of the contralateral SB response was due to the callosotomy and not to the loss of scopolamine's effectiveness, several subjects received a second infusion of scopolamine at the end of the experiment; no change in SB responding was observed.



**Figure 4. Callosotomy reinstates contralateral evoked spindle-bursts (SBs) after scopolamine infusion.** (A) Depiction of experimental procedure in infant rats ( $n = 6$ ) showing the three 15-min recording periods: After a baseline period, scopolamine was infused into the left S1, followed by callosotomy. During each period, responses of the left and right S1s were recorded in response to plantar surface stimulation of the right forepaw. (B) Mean percentage of right forepaw plantar surface stimulations producing associated SBs. During the baseline period, only contralateral SBs were produced. After scopolamine infusion into the left S1, ipsilateral responses predominated (red bar). Subsequent callosotomy reinstated contralateral responding.  $\dagger P < 0.0001$  in relation to right hemisphere. Mean  $\pm$  s.e. (C) Representative evoked SBs in left (purple) and right (green) S1s in response to right forepaw plantar surface stimulations (denoted by arrows) in a P3 subject. Contralateral responding during the baseline period was followed by ipsilateral responding after scopolamine infusion and reinstatement of contralateral responding after subsequent callosotomy. The higher oscillation frequency of one of the post-callosotomy SBs is indicated.

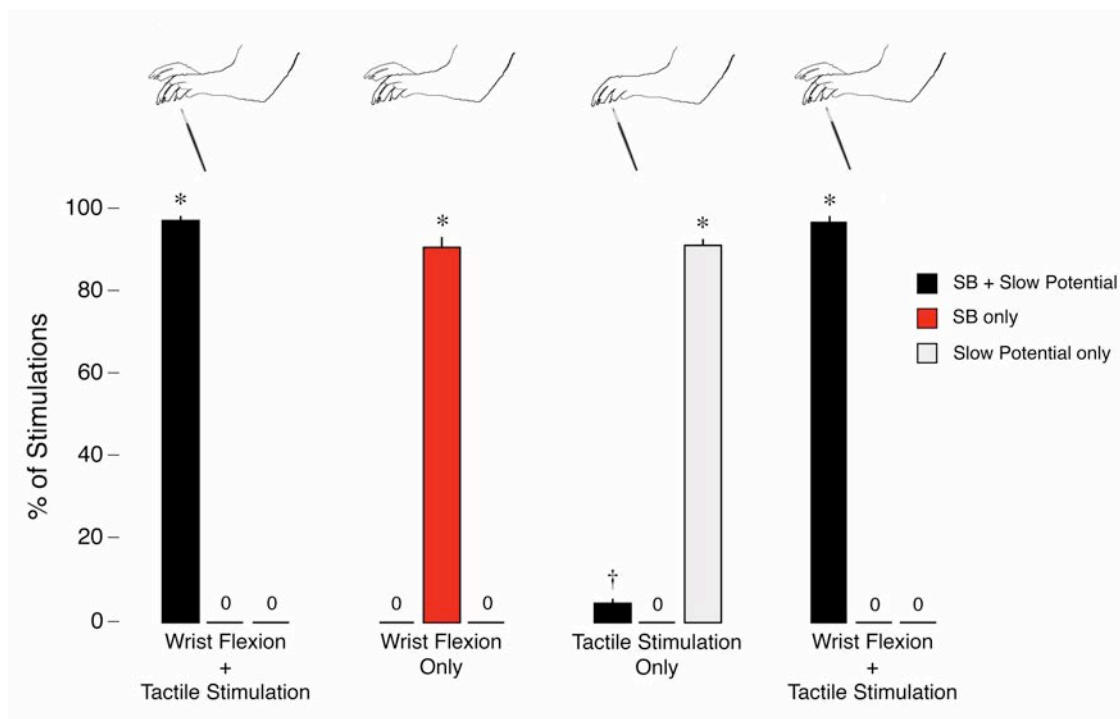
Although contralateral SB responses were reinstated by callosotomy, the SBs that were reinstated were not expressed with their characteristic oscillation frequency. Specifically, during the baseline period, mean evoked SB frequency in the left S1 after right forepaw stimulation was  $17.7 \pm 0.3$  Hz. After scopolamine infusion, mean evoked SB frequency in the right S1 after right forepaw stimulation did not change significantly ( $17.5 \pm 0.4$  Hz;  $t_{10} = 0.3$ , NS). In contrast, mean evoked SB frequency in the left S1 to right forepaw stimulation nearly doubled after callosotomy,  $34.0 \pm 0.7$  Hz, which is significantly greater than both previous conditions ( $t_{10s} > 28.8$ ,  $P_s < 0.0001$ ).

#### Dissociating tactile and proprioceptive stimulation

Given that bupivacaine blocked the expression of SBs without affecting expression of the slow potential, we hypothesized that the two responses could be dissociated by taking care to provide only tactile or proprioceptive stimulation to the forepaw. This hypothesis arose from our observation that the standard stimulus used here comprised tactile stimulation of the plantar surface along with dorsiflexion at the wrist. Therefore, in 8 additional P3-4 rats, subjects were prepared as in previous experiments and then presented with a succession of stimuli. As shown in Figure 5, the standard stimulus—wrist dorsiflexion with tactile stimulation—resulted in the expected SBs embedded within slow potentials ( $96.9 \pm 1.2\%$ ). When wrist dorsiflexion alone was presented, only SBs were detected in the contralateral S1 ( $90.6 \pm 2.5\%$ ). And when tactile stimulation alone was presented, the predominant response was a slow potential without an SB ( $90.9 \pm 1.3\%$ ); embedded SBs were only occasionally produced ( $3.7 \pm 0.7\%$ ), presumably due to the difficulty of completely eliminating wrist dorsiflexion. It



should also be noted that a control procedure involving flexion at the elbow joint never produced activity in the contralateral S1 (data not shown). A final control period of wrist flexion with tactile stimulation again produced the expected embedded SBs in the contralateral S1 ( $96.7 \pm 1.1\%$ ).



**Figure 5. Dissociation of tactile and proprioceptive S1 responses in newborn rats.** Top: Illustrations depicting the four types of stimulation applied to the left or right forepaw in successive 15-min periods: wrist dorsiflexion with tactile stimulation; wrist dorsiflexion alone; tactile stimulation alone; and another period of wrist dorsiflexion with tactile stimulation. Bottom: percentage of forepaw stimulations that, in the contralateral S1, produced SBs embedded within slow potentials, SBs only, or slow potentials only. A fourth stimulation type, not shown here and comprising flexion of the elbow joint, never resulted in contralateral S1 activity. \* significant difference from the other two response categories ( $p < 0.0001$ ). † significant difference from the 'SB only' response category ( $p < 0.001$ ). Mean + s.e.

## Discussion

We have revealed mutually inhibitory interactions between primary somatosensory cortical areas mediated by the corpus callosum. This functional inhibition was immediately evident upon examination of spontaneous, active sleep-related SBs after the corpus callosum was severed: specifically, the rate and oscillation frequency of SBs increased dramatically after callosotomy, and SBs in the two hemispheres occurred in much closer temporal proximity to each other. Thus, with regard to the ongoing debate concerning the relative importance of excitatory and inhibitory functions of the corpus callosum (Cook, 1984; Denenberg et al., 1986; Koralek & Killackey, 1990; Reggia et al., 2001; Bloom & Hynd, 2005), it seems clear that in newborn rats each hemisphere functionally inhibits the other via callosal projections. Whether these projections achieve this functional inhibition through local inhibitory or excitatory postsynaptic effects remains unclear.

It may seem paradoxical that SBs were produced in closer temporal proximity between the two hemispheres in callosotomized subjects (see Figure 2). As already mentioned, it is most likely that this apparent increase in interhemispheric synchrony resulted from the loss of callosal inhibition, thereby increasing the likelihood that a limb twitch would trigger an SB. This is likely the case, as SB production occurs in response to sensory feedback from the periphery and myoclonic twitches are expressed in bouts comprising contemporaneous but non-simultaneous movements of individual limbs (Robinson et al., 2000). The precise relationship among limb movements, sensory feedback, and SB production is still unknown.

Unilateral infusions of the muscarinic receptor antagonist, scopolamine,

provided further support for the hypothesis that the corpus callosum functionally inhibits left and right S1s in newborn rats. Under normal conditions where SBs were evoked by stimulation of the plantar surface of a forepaw, only contralateral responses were observed. However, when scopolamine was infused unilaterally into the left S1 and the right forepaw was stimulated, contralateral SB responses were significantly reduced, thus suggesting that excitatory muscarinic activation is necessary to produce consistent contralateral SB responses to peripheral stimulation. In addition to inhibiting contralateral SBs, scopolamine infusion disinhibited ipsilateral SBs, an unexpected finding that suggests the presence of representations of each forepaw in both hemispheres.

Although it appeared to us initially that scopolamine had disrupted the ability of the left S1 to produce SBs, this was clearly not the case as subsequent callosotomy reinstated their expression (although at a higher oscillation frequency). Thus, both the inhibition of the contralateral response and the disinhibition of the ipsilateral response must rely on callosal modulation of S1 activity. Interestingly, with respect to motor behavior, alterations in interhemispheric balance in humans—as occur with asymmetric parkinsonism and other neurological disorders—can result in the disinhibition of ipsilateral pathways and the production of “mirror movements” (Li et al., 2007b).

Too little is currently known about cortical circuitry and callosal connectivity, especially during the early postnatal period, to provide a realistic model of the mechanisms responsible for the cholinergically mediated disinhibition of SBs in the ipsilateral S1 or the increase in SB oscillation frequency after callosotomy. Any such model will need to take into account the neonate’s rapidly changing cortical circuitry, as well as the contributions that the cortical

subplate and other transient structures might make to neurophysiological activity during the early postnatal period when the callosal fibers are establishing topographic connections (Wise & Jones, 1976; Innocenti & Price, 2005a).

As shown here, SBs evoked by plantar surface stimulation were typically embedded within large, slow potentials. However, these two responses to sensory stimulation are dissociable, as the local anesthetic, bupivacaine, effectively blocked expression of SBs without affecting the slow component. We examined this issue further through specific tactile, proprioceptive, or combined stimulation of the forepaw. Whereas plantar surface stimulation with wrist dorsiflexion produced the expected embedded SB response, wrist dorsiflexion alone produced only SB responses; the predominant response upon specific tactile stimulation was the slow potential without an SB.

Spontaneous SBs were rarely embedded within slow potentials. This could reflect the fact that, in the present experiment, pups were suspended in a prone position such that the limbs did not make contact with any surface. Therefore, these test conditions allowed for proprioceptive, but not tactile, feedback from twitching limbs. Under more natural conditions where limbs can make contact with other objects, including littermates, we might expect twitching to result in both tactile and proprioceptive feedback and, consequently, embedded SBs.

The continued detection of spontaneous SBs in the left S1 after bupivacaine and scopolamine infusion was surprising. Because sleep-related twitching can produce SBs in multiple areas of S1 contemporaneously, it is possible that travelling waves from nearby cortical tissue obscured the effects of

bupivacaine on local SB production; such travelling SBs have been demonstrated in newborn rats (Khazipov et al., 2004). In contrast, SBs would not be expected to travel from nearby tissue when evoked through specific peripheral stimulation. In light of the fact that scopolamine disinhibited SB activity in the right S1 in response to ipsilateral forepaw stimulation, we might have expected a doubling of spontaneous SBs in the right S1 as a result of the combined sensory input from twitches of the contralateral and ipsilateral forepaws. That we did not observe any increase in the occurrence of spontaneous SBs after scopolamine infusion cannot be attributed to the obscuring effects of travelling waves.

There is an alternative explanation for the differential effects of bupivacaine and scopolamine on spontaneous and evoked SBs. Specifically, it is possible that spontaneous SBs triggered by the sensory feedback from self-generated myoclonic twitches recruits different cortical mechanisms than SBs evoked by externally applied peripheral stimulation. Such a possibility is further supported by the finding that callosotomy doubled the expression of spontaneous SBs but decreased by 30% the likelihood of evoked SBs to contralateral forepaw stimulation. Confirming and elucidating this distinction between spontaneous and evoked SBs will be important for understanding the possible function of sleep-related twitching for sensorimotor development.

Sensory feedback during twitching has been hypothesized to play a role in the self-organization of spinal cord circuits that support the nociceptive withdrawal reflex (Pettersson et al., 2004; Schouenborg, 2008). This reflex, which entails the integration of specific sensory fields in the paw with functionally relevant motor commands, develops over the first few postnatal weeks in rats (Holmberg & Schouenborg, 1996). In that context, it is perhaps surprising that

the two separable but functionally related features of distal limb stimulation used here—wrist dorsiflexion and plantar surface stimulation—result in integrated activity in somatosensory cortex so soon after birth in rats, and that they can also be transferred together across the corpus callosum to the contralateral region. Such early expression of these phenomena suggests that spontaneous movements in fetuses, which are phenomenologically similar to those expressed in neonates (Robinson et al., 2000), also elicit sensory feedback and nervous system activation that could contribute to the development of neural circuits.

If SBs and myoclonic twitching jointly contribute to the self-organization of the sensorimotor system (Khazipov et al., 2004; Seelke et al., 2005b), then it is possible that interference with callosal functioning—as occurs with callosotomy—would obscure the temporal relationship between individual movements and their associated SBs, thereby disrupting map formation. Such a situation could prevail in humans with AgCC or other disorders that entail disruption of callosal functioning (Paul et al., 2007), as well as humans who have experienced callosotomy at an early age (Lassonde et al., 1991).

**CHAPTER III**  
**AN ABRUPT DEVELOPMENTAL SHIFT IN CALLOSAL MODULATION OF SLEEP-RELATED SPINDLE BURSTS COINCIDES WITH THE EMERGENCE OF EXCITATORY-INHIBITORY BALANCE AND A LOSS OF SOMATOSENSORY CORTICAL PLASTICITY: DISINHIBITION OF SLEEP-RELATED SPONTANEOUS SPINDLE BURSTS AS A BIOASSAY OF SOMATOSENSORY CORTICAL PLASTICITY**

As described in the previous chapter above, transecting the corpus callosum (CCx) of postnatal day (P)1-6 rats disinhibits spontaneous SBs in the forelimb region of S1 during periods of active sleep-related myoclonic twitching (Marcano-Reik & Blumberg, 2008). CCx also disrupts evoked S1 responses to peripheral stimulation of the contralateral forepaw. These findings suggest that the corpus callosum – soon after birth and at least through P6 – exerts a net inhibitory influence over homotopic cortical circuits and plays a modulatory role in sensory processing.

Models of cortical injury and recovery typically involve direct damage to specific areas within cortical lobes (Kolb et al., 2010). Such studies have revealed that the long-term consequences of different types of cortical damage depend on the stage of brain development at the time of injury. For example, in rats, functional recovery is best when cortical injury is produced during the second postnatal week in relation to the first postnatal week, that is, after neuronal migration to superficial layers is complete around P6. However, if SBs contribute to the development of somatosensory representations in S1, as has been suggested (Khazipov et al., 2004), then the dramatic CCx-induced disinhibition of SB activity observed before P6 might indicate a state of heightened plasticity.

Here we investigated developmental changes in CCx-induced disinhibition and its association with somatosensory cortical plasticity. We document a sudden disappearance of CCx-induced disinhibition, accompanied



by molecular and cellular changes that are associated with a loss of cortical plasticity after P6. We propose that CCx in early development offers a new and unique model system for examining the effects of early perturbations of cortical circuitry on somatosensory processing and recovery of function. All together, these findings support the hypothesis that myoclonic twitches serve as a primary source of sensory input driving activity-dependent processes in somatosensory cortex during early postnatal development, similar to the role played by retinal waves in the development of visual cortex (Katz & Shatz, 1996; Wong, 1999).

### **Materials and methods**

All experiments were performed in accordance with National Institutes of Health guidelines for the care and use of animals in research and were approved by the Institution Animal Care and Use Committee of the University of Iowa. All efforts were made to minimize the number of animals used.

For this study, mothers and their litters were housed in standard laboratory cages (48 x 20 x 26 cm) in the animal colony at the University of Iowa. Food and water were available ad libitum and all animals were maintained on a 12-hour light-dark cycle with lights on at 0700 hours. All experiments took place during the lights-on period. Litters were culled to 8 pups within 3 days of birth (day of birth = P0). For all subjects tested, there were no differences observed in body weight, appearance, or behavior (t tests were performed to confirm). Daily observations were conducted to ensure that all subjects maintained a visible milk band, which reflects recent feeding and appropriate maternal care.

Developmental changes in cortical activity

Subjects. A total of 32 pups from 32 litters were used. Subjects were tested on P2, P4, P6, P7, P8, P9, P12, or P15 (n = 4 per age). Males and females were equally represented among the subjects.

Surgery. On the day of testing, 2 littermates underwent CCx or sham surgery (with counterbalancing of the order of surgery). These procedures are identical to those described in the previous chapter and a previously published report (Marcano-Reik & Blumberg, 2008). Under isoflurane anesthesia, a 2-3 mm opening was created in the skull, halfway between bregma and lambda and parallel and lateral to midline. In CCx subjects, a thin surgical knife was inserted to a depth of approximately 5 mm and was swept in an anterior-to-posterior direction to transect the corpus callosum. Sham surgeries were identical except that the surgical knife was not inserted. In all subjects, 2 recording sites were prepared for placement of cortical surface electrodes over the left and right S1s. Each site consisted of 2 holes separated by approximately 2 mm and centered over the forelimb region of S1, approximately 1 mm rostral to bregma and 2-3 mm lateral to midline.

As described previously (Karlsson et al., 2005; Marcano-Reik & Blumberg, 2008), a custom-built stainless steel apparatus, designed to attach to the earbar holders of a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, United States), was secured to the skull with cyanoacrylate adhesive. Bipolar stainless steel electrodes (50  $\mu$ m diameter, California Fine Wire, Grover Beach, CA) were inserted into the left and right nuchal muscles. A ground wire was implanted anterior to the nuchal EMGs. Electrodes were secured with flexible collodion. At the end of surgery, the pup's trunk was lightly wrapped in gauze while the limbs remained exposed. These surgeries lasted approximately 10

minutes. The pups were then placed in a humidified incubator maintained at thermoneutrality (35°C) to recover for 1 hour. At the end of the recovery period, pups were transferred to an electrically shielded chamber for testing.

Procedure. As reported in the previous chapter and a previously published report (Marcano-Reik & Blumberg, 2008), the pup's head was fixed in the stereotaxic apparatus and its ventrum was placed on a flat support bar with its forelimbs and hindlimbs dangling freely on both sides without contacting any surface. Temperature-controlled water flowing through a concave double-walled glass chamber, situated beneath the pup, helped to control the thermal environment and, in conjunction with a heat lamp, maintain the pup's brain temperature at 37°C throughout testing. Custom-made Ag/AgCl electrodes were inserted just below the cortical surface into the pre-drilled recording sites. Ground electrodes were placed in the cerebellum. Electroencephalographic (EEG) and electromyographic (EMG) electrodes were connected to differential amplifiers (A-M Systems, Carlsborg, WA; filter setting: 0.1-3000 Hz; amplification: x10,000). Neural and EMG signals were sampled at 12.5 kHz using a digital interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Once sleep-wake cyclicity was observed, both behaviorally and electrophysiologically, the plantar surface of the left and right forepaws was stimulated with a fine brush, typically eliciting dorsiflexion at the wrist joint. Electrode placements within S1 were judged successful when plantar surface stimulation of the contralateral forepaw resulted in an evoked SB embedded within a slow potential. Other parts of the limb and body were also stimulated to assure the specificity of the response and recording location.

For all subjects, after at least one hour of acclimation in the stereotaxic apparatus, after which time sleep-wake behaviors and neurophysiologic activity were evident, the recording session began with a 15-minute period of behavioral scoring (twitching and wake-related behaviors), and was followed by a 15-minute period of plantar surface forepaw stimulation. EMG and EEG data were acquired continuously throughout the test. In all subjects, visual observation and EMG activity in the left and right nuchal muscles provided measures of behavioral state and myoclonic twitching (Karlsson et al., 2005; Seelke & Blumberg, 2005; Marcano-Reik & Blumberg, 2008). Consistent with previous reports (Gramsbergen et al., 1970a; Karlsson et al., 2005), myoclonic twitches of the limbs and tail, indicative of active sleep, were defined as phasic, rapid, and independent movements. High-amplitude movements, indicative of wakefulness, included locomotion, stretching, and yawning. In addition, during periods of sleep-wake observation and forepaw stimulation, the experimenter marked the events in synchrony with the electrophysiologic records by pressing a key on the keyboard during data acquisition.

**Histology.** Upon completion of testing, all CCx pups were overdosed with an intraperitoneal injection of sodium pentobarbital and perfused transcardially with phosphate-buffered saline, followed by a 3% formalin solution. Brains were postfixed for at least 48 hours in a formalin solution before being sliced in the coronal plane (50  $\mu$ m sections). Light microscopy was then used to assess the extent of damage to the corpus callosum. In this experiment, approximately 90% of the corpus callosum was destroyed, beginning at the anterior portion and extending posteriorly.

**Data Analysis.** For each subject, 15-minute periods of synchronized data

comprising digital records of behavior, nuchal EMG activity, and EEG activity were created and analyzed using Spike2 software, as described previously (Marcano-Reik & Blumberg, 2008). For all analyses of sham and CCx littermate subjects, paired (within-subjects) t tests were performed using JMP 5.0 software (SAS, Cary, North Carolina, USA). For all tests, alpha was set at 0.05 and Bonferroni corrections were used to correct alpha for multiple comparisons.

Myoclonic twitches were counted by referring to both the behavioral record (i.e., limb and tail twitching) and the left and right nuchal EMG activity. Thresholds were set in Spike2 to analyze nuchal EMG activity (Mohns & Blumberg, 2008). Cursors were used to identify individual twitches in the EMG records and relate them to behaviorally scored twitches. A memory buffer was created with all the marked twitches, and an analysis was run to count all the events during the 15-minute recording period.

Spontaneous and evoked SBs were identified by referring to both the raw EEG records of the left and right S1s as well as filtered records (band-pass: 5-40 Hz). Using criteria identical to those described previously (Khazipov et al., 2004; Marcano-Reik & Blumberg, 2008), SBs were defined as comprising at least 3 complete oscillations, > 100 ms in duration, and containing at least 1 oscillation that exceeded 50  $\mu\text{V}$  in amplitude (from baseline to peak). In contrast with spontaneous SBs, evoked SBs were embedded within large, slow potentials with amplitudes  $\geq 100 \mu\text{V}$ . SB oscillation frequency was also analyzed in sham and CCx subjects as described in the previous chapter and in a previously published report (Marcano-Reik & Blumberg, 2008). Briefly, the frequency (in Hz) of an individual SB was determined by averaging two successive wavelengths and then calculating the reciprocal. Mean SB frequency was determined for each

subject by averaging across 20 individual SBs, randomly selected across the 15-minute record.

SB latencies were measured and compared for all subjects, as described in the previous chapter and in a previously published report (Marcano-Reik & Blumberg, 2008). For this analysis, 20 'anchor' SBs in the left S1 recording were selected at random and its duration measured. Then, the latencies between the anchor SB and the prior ( $L^-$ ) and subsequent ( $L^+$ ) SBs in the right S1 recording were determined (see Chapter II, Figure 2C). Mean values of  $L^-$  and  $L^+$  were always statistically indistinguishable. SB-to-twitch ratios were calculated to estimate the likelihood that an SB was expressed in response to a twitch. For this measure, we determined the total number of spontaneous SBs during the 15-minute period of behavioral scoring in the left S1, and divided this number by the total number of twitches over the same 15-minute period for that subject.

#### Effect of GABAergic agonists and antagonists on SBs

**Subjects and surgical preparation.** A total of 32 P4 rats from 8 litters were used. On the day of testing, 2 littermates experienced sham surgery and 2 additional littermates received CCx surgery under isoflurane anesthesia. All subjects were prepared for testing as described above ( $n = 8$  per group). Surgeries were staggered to minimize the latency between surgery and testing.

**Procedure.** After 1 hr of recovery from surgery, the pup was transferred to the recording apparatus. After 1 hr of acclimation, the recording session began with a  $1\mu\text{l}$  infusion of the vehicle, artificial cerebrospinal fluid (ACSF), into the left or right S1 and behavior was scored for 15 minutes immediately following the infusion. After the behavioral recording period, the subject received either a

1 $\mu$ l infusion of the GABA<sub>A</sub> receptor agonist, muscimol hydrobromide (0.01M mixed with ACSF; Sigma-Aldrich, St. Louis, MO, USA), or a 0.5 $\mu$ l infusion of the GABA<sub>A</sub> antagonist, bicuculline methiodide (10 $\mu$ M; Sigma-Aldrich, St. Louis, MO, USA). The volumes and concentrations for muscimol (Campolattaro & Freeman, 2008) and bicuculline (Jones & Barth, 2002) are based on previously published work. After the infusion, another 15-minute period of behavioral scoring was conducted. The remaining 3 littermates were tested in succession and the order of testing (i.e., sham vs. CCx; muscimol vs. bicuculline; left vs. right S1) was randomized.

Infusions were delivered using a Hamilton microliter syringe with a 25-gauge needle (Model 7001, Hamilton, Reno, NV, USA) secured to the stereotaxic apparatus and mounted directly above the infusion site. The syringe was lowered just below the cortical surface into a pre-drilled hole located halfway between the two Ag/AgCl electrode sites. The infusion rate for all infusions was 0.1  $\mu$ l/s. These methods are identical to a previous report (Marcano-Reik & Blumberg, 2008) and it has been shown before that surface and intracortical infusions do not differ significantly in their effects on SB activity (Hanganu et al., 2007).

Three of the muscimol subjects received an infusion of the same concentration of fluorescently labeled muscimol (BODIPY TMR-X conjugate; Invitrogen, Eugene, Oregon, USA; Allen et al., 2008). At the end of the recording session, the pups were overdosed with an intraperitoneal injection of sodium pentobarbital and perfused transcardially with phosphate-buffered saline, followed by a 3% formalin solution. Brains were post-fixed for at least 48 hours in a formalin solution before being sliced in the coronal plane with a sliding

microtome (50  $\mu\text{m}$  sections). A fluorescent microscope was used to visualize the distribution of muscimol throughout the brain.

Data Analysis. For all subjects, paired (within-subjects) t tests were performed using JMP 5.0 software (SAS, Cary, North Carolina, USA). For all tests, alpha was set at 0.05 and Bonferroni corrections were used to correct alpha for multiple comparisons.

#### K<sup>+</sup>Cl<sup>-</sup> co-transporter 2 (KCC2) Western blot analysis

Procedure. Cortical tissue including the S1 region of P5, P6, P7, P8, and P9 rats (n = 2 per age) was homogenized in a buffer solution (150 mM NaCl, 1% Triton X-100, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris-HCl, pH 8.0). Samples were incubated in buffer (80 mM Tris-HCl, 2% SDS, 10% glycerol, 5.3%  $\beta$ -mercaptoethanol, 0.025% bromophenol blue) at 75°C for 10 minutes. Samples were run, 10  $\mu\text{g}$  each, on a 7.5% polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was blocked in TBST (Tris Buffered Saline, 0.1% Tween-20) and 5% milk, then incubated overnight at 4°C with antibodies against KCC2 (Rabbit anti-KCC2, Upstate Cell Signaling Solutions, Lake Placid, NY; 1:1000 dilution in TBST) and  $\beta$ -tubulin (Mouse anti- $\beta$ -tubulin, Sigma-Aldrich, St. Louis, MO; 1:1000 dilution in TBST). The membrane was washed in TBST, and probed with corresponding HRP-conjugated secondary antibodies (Goat anti-Rabbit HRP IgG HL, Jackson ImmunoResearch, West Grove, PA; 1:10,000 dilution in TBST with 5% milk; Goat anti-Mouse HRP IgG HL, Pierce Biotechnology, Rockford, IL; 1:5000 dilution in TBST with 5% milk) and imaged using SuperSignal West Pico Chemiluminescent Substrate (Pierce Biotechnology, Rockford, IL; Garrett, 2009).



Quantification: Scanned images of blots were quantified using NIH Image/J and the “Analyze Gels” functions. KCC2 bands were quantified for each lane and normalized to the b-tubulin signal, and data were expressed as arbitrary units (with the normalized P5 signal set as “1”).

#### Recovery of function after callosotomy

Subjects and surgical preparation. A total of 144 P1-P15 infant rats from 24 litters were tested. Littermates received sham or CCx surgery at P1, P6, or P8. A total of 60 littermates received surgery at P1 and were tested at P1, P2, P4, P6, or P8 (weights did not significantly differ between sham and CCx subjects at P8; sham subject weights ranged from 20.2-24.1g; CCx subject weights ranged from 19.1-24.5g). A total of 36 littermates received surgery at P6 and were tested at P6, P9, or P13 (weights did not significantly differ between sham and CCx subjects at P13; sham subject weights ranged from 30.6-36.9g; CCx subject weights ranged from 30.5-40.2g). Finally, a total of 48 littermates received surgery at P8 and were tested at P9, P12, or P15 (data from the identically treated and tested P8 subjects described above were added to this dataset; weights did not significantly differ between sham and CCx subjects at P15; sham subject weights ranged from 37.4-42.7g; CCx subject weights ranged from 38.9-43.2g). At each age, 6 sham and 6 CCx littermates from 6 litters were tested. Males and females were equally represented among the subjects.

Surgeries were identical to those described above. However, for those subjects that received sham or CCx surgeries at P1, P6, or P8 and were to be tested at a later age, the incision was closed with Vetbond and pups, along with their remaining littermates, were transferred to a humidified incubator

maintained at thermoneutrality (35°C) to recover for 1 hour. At the end of the recovery period, all pups, with the exception of the 2 subjects to be tested that same day, were placed back in the home cage with their mother and returned to the colony. At the appropriate time, 2 littermates were transferred to an electrically shielded chamber for acclimation and neurophysiological and behavioral testing, as described above. All procedures for recording evoked and spontaneous activity were the same as described above.

Data Analysis. For all subjects, paired t tests were performed at each age using JMP 5.0 software (SAS, Cary, North Carolina, USA). For all tests, alpha was set at 0.05 and Bonferroni corrections were used to correct alpha for multiple comparisons.

### Immunofluorescence

Procedure. To determine normal changes in presynaptic terminals and receptor populations, additional subjects were anesthetized with isoflurane and received sham surgery (i.e., the same surgical procedures as those described above) on P6, P7, P8 or P9 (n = 3 at each age). Subjects were perfused transcardially with cold 0.1M phosphate buffered saline (PBS) followed by 2% paraformaldehyde in PBS (pH = 7.4). Following perfusion the brains were extracted and post-fixed in cold 2% paraformaldehyde in PBS overnight. The brains were then cryoprotected in 30% sucrose in PBS at 4°C and frozen in OCT compound (Tissue-Tek; Sakura-Finetek) using dry ice/ethanol-cooled isopentane.

Coronal cryostat sections (12  $\mu$ m) at the level of the corpus callosum were cut on a Leica CM1850 cryostat and collected with the aid of a Leica Cryo tape

transfer system. Tissue sections were air-dried and then blocked in 2.5% bovine serum albumin, 0.1% Triton X-100 in PBS for 1 hour, followed by overnight incubation at 4°C with primary antibodies: guinea pig anti-vesicular glutamate transporter-1 (VGLUT-1; 1:500, Chemicon) and mouse anti-glutamic acid decarboxylase (GAD; mAb GAD-6; 1:10 Developmental Studies Hybridoma Bank) diluted in the same blocking solution. Sections were washed in PBS and incubated for 1 hour at room temperature with the appropriate secondary antibodies conjugated to Alexa 488 and 568 (Invitrogen). Sections were washed in PBS containing a counterstain (4', 6' diamidino-2 phenylindole) and mounted in Gel/Mount (Biomedica) aqueous mounting media.

**Image Acquisition and Analysis.** Quantification of presynaptic (VGLUT-1-positive or GAD-positive) puncta was performed on 6 sections per animal from 3 different animals at each time point (i.e., 18 fields for each marker). Images were taken of fields within S1 cortex using 63X Plan Apo objectives on a Leica DM5000B digital epifluorescence microscope. Digital images were captured in Adobe Photoshop and similarly adjusted for brightness and contrast. Images were thresholded in NIH Image/J and synaptic puncta were counted by using the Analyze Particle module; the accuracy of these automated quantifications was confirmed in a portion of each field by manual counts.

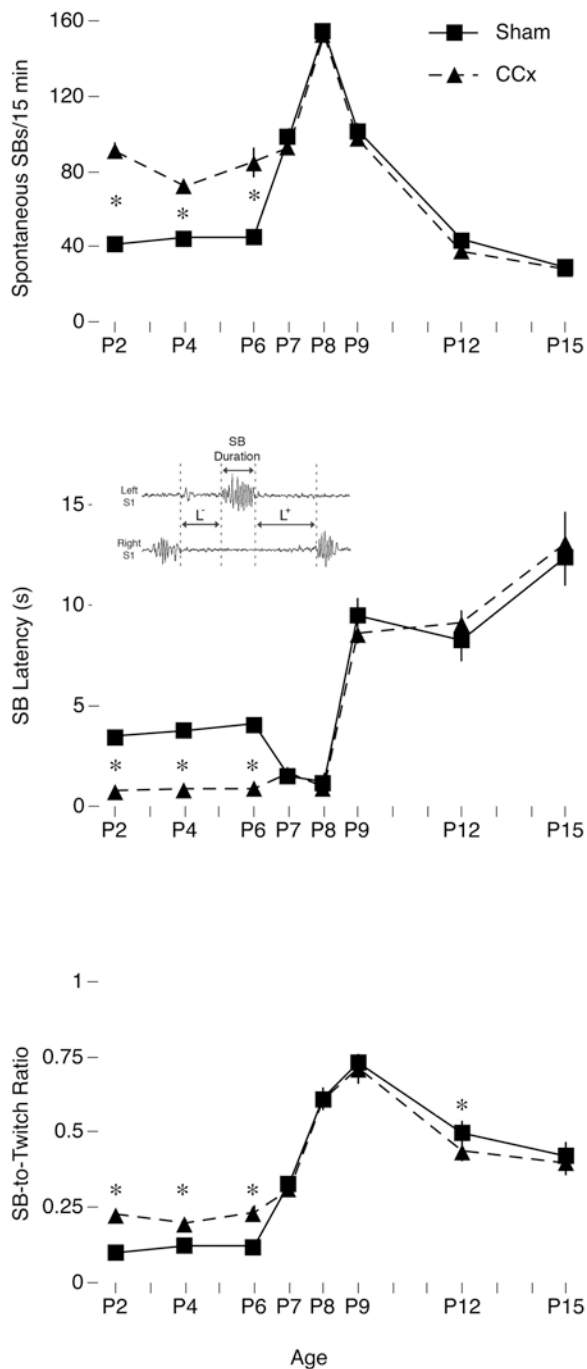
**Data Analysis.** To control for field-by-field variations in cell density, we normalized the number of presynaptic terminals to the number of cell nuclei in each field. The proportion of cells that labeled with antibodies to the neuronal marker NeuN was also monitored, and did not change between P6 and P9 (data not shown), confirming that total cell density is proportional to neuronal density. ANOVAs were separately conducted for each marker (i.e., VGLUT-1 and GAD)

and cortical depth (i.e., layers 2-3 and 4-6) using JMP 5.0 software (SAS, Cary, North Carolina, USA). Student's t test was used for post hoc comparisons. Alpha was set at 0.05 and the Bonferroni correction procedure was used.

## **Results**

### **Abrupt disappearance of CCx-induced disinhibition**

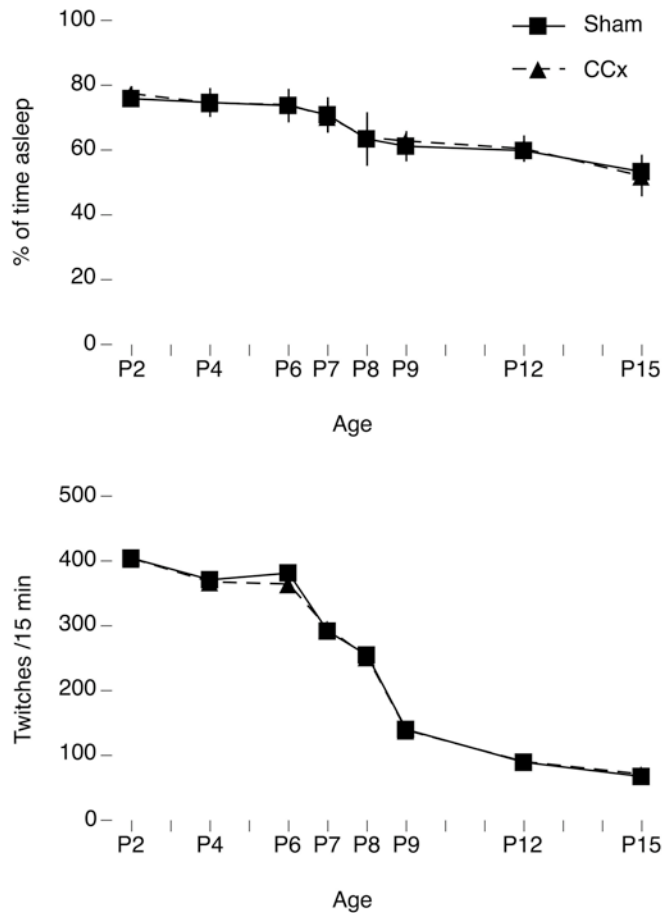
As shown in the previous chapter and in a previously published report (Marcano-Reik & Blumberg, 2008), CCx doubled the rate of spontaneous SBs in P1-6 subjects (Figure 6, Top:  $P_s < 0.001$ ,  $n = 4/\text{age}$ ). This disinhibition was reflected in shorter latencies separating SBs between the two hemispheres (Figure 6, middle:  $P < 0.0001$ ) as well as increases in the SB-to-twitch ratio (Figure 6, bottom;  $P < 0.001$ ). Thus, it appears that CCx increased the probability that a twitch would trigger an SB in the contralateral S1. It should also be noted that, similar to earlier findings (Marcano-Reik & Blumberg, 2008), SB oscillation frequency increased significantly after CCx, but SB duration was unaffected (data not shown).



**Figure 6. Changes in SB activity in sham and callosotomized (CCx) P2-15 rats.** Top Panel: Rate of spontaneous SBs doubles after CCx at or before P6. Middle Panel: CCx-induced disinhibition is accompanied by decreased interhemispheric SB latencies. Inset: Illustration of method used to score SB latencies; only mean L-values presented. Bottom Panel: CCx-induced disinhibition is accompanied by an increase in SB-to-twitch ratio. \* significant difference between groups.

Beginning at P7, CCx no longer disinhibited spontaneous SBs in relation to shams (Figure 6, Top). Coincident with this disappearance of CCx-induced disinhibition, the rate of SBs in both sham and CCx subjects increased sharply and peaked at P8, steadily decreasing over the next several days. SB latencies and SB-to-twitch ratios mirrored these developmental changes. All together, these observations indicate the emergence after P6 of excitatory-inhibitory balance that likely reflects changes in the intrinsic cortical circuitry that produces SBs and in the modulatory influence exerted by the corpus callosum.

We next confirmed that the effects of CCx described above were not due to alterations of sleep or rates of twitching. Given that disconnection of the cortex does not alter sleep-wake cycling in P8 rats (Karlsson et al., 2004), we expected CCx to have no effect on the percentage of time asleep in relation to shams; indeed, no effect was found (Figure 7, top). Also, since twitches are produced within the brainstem (Kreider & Blumberg, 2000; Karlsson & Blumberg, 2005; Karlsson et al., 2005), we anticipated that CCx would not alter or influence their occurrence, which is what was observed (Figure 7, bottom). Interestingly, we found that over several days beginning at P6, the rate of twitching rapidly declined in both sham and CCx subjects.



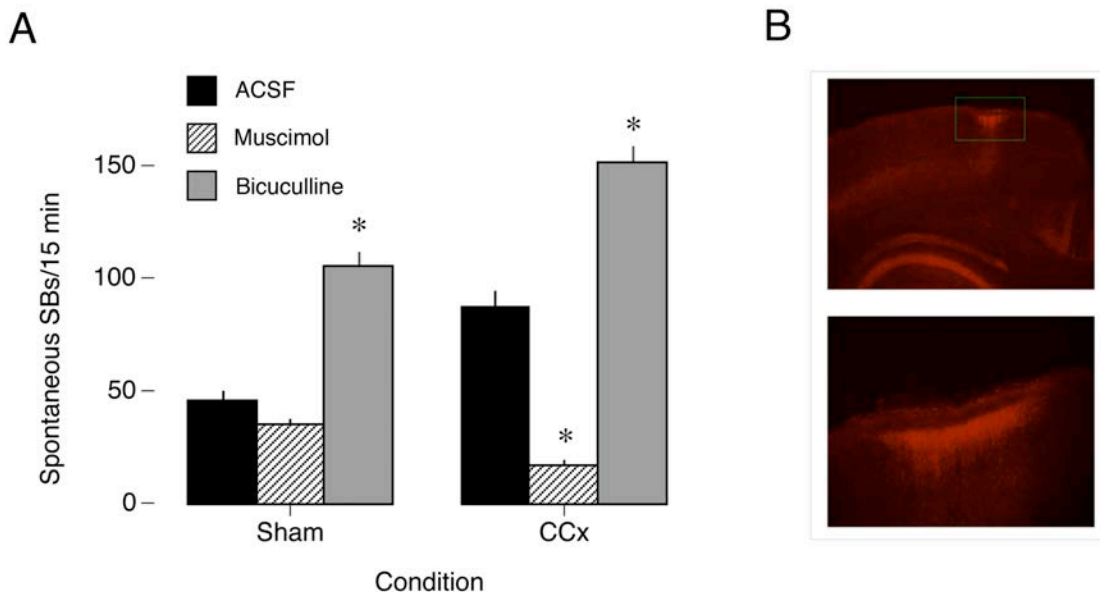
**Figure 7. Developmental changes in percentage of time asleep and rates of active sleep-related twitching in sham and callosotomized (CCx) P2-15 rats.** Top: In relation to shams, CCx had no effect on the percentage of time asleep across the 15-min recording period at any age. Bottom: Although the quantity of nuchal muscle twitches per 15 min decreased with age, especially after P6, CCx again had no effect on twitching at any age. Because twitches are produced within the brainstem, we anticipated that CCx would not influence their occurrence. Means + sem.

### GABAergic modulation of spontaneous SBs

It was hypothesized that CCx-induced disinhibition of SBs through P6 results from the loss of excitatory drive onto GABAergic interneurons. To test this hypothesis, unilateral infusions of artificial cerebrospinal fluid (ACSF) were administered just below the S1 cortical surface in P4 rats, followed by an infusion of the GABA<sub>A</sub> agonist muscimol or antagonist bicuculline (n = 8 subjects/group). In shams, muscimol did not significantly influence spontaneous SB production whereas bicuculline significantly increased spontaneous SBs ( $P < 0.001$ , n = 8) across the 15-minute test period in relation to ACSF (Figure 8A). On a percentage basis, muscimol decreased SBs by only 11.3% whereas bicuculline increased SBs by 60% in relation to ACSF.

If the corpus callosum exerts its effects at P4 by preferentially exciting GABAergic interneurons, then it should be possible to compensate for the loss of GABAergic modulation after CCx by infusing muscimol. This is exactly what was found. Specifically, in relation to ACSF, muscimol and bicuculline significantly reversed and exacerbated ( $P_s < 0.001$ , n = 8), respectively, the effect of CCx-induced disinhibition on spontaneous SBs. On a percentage basis, muscimol decreased SBs by 71% and bicuculline increased SBs by 64.5% in relation to ACSF (Figure 8A).





**Figure 8. (A) Effect of unilateral infusion of ACSF, muscimol, or bicuculline into S1 on spontaneous SB activity in sham and CCx P4 rats.** Note that in CCx subjects, but not sham subjects, muscimol and bicuculline have equal and opposite effects on SB production. \*significant difference from ACSF. Means + sem. (B) The distribution of fluorescently labeled muscimol (0.01M) after a 1  $\mu$ l infusion into the left S1 of a P4 rat. Top: Coronal section of the left hemisphere showing the site of muscimol infusion (green box). Bottom: The boxed area above is magnified.

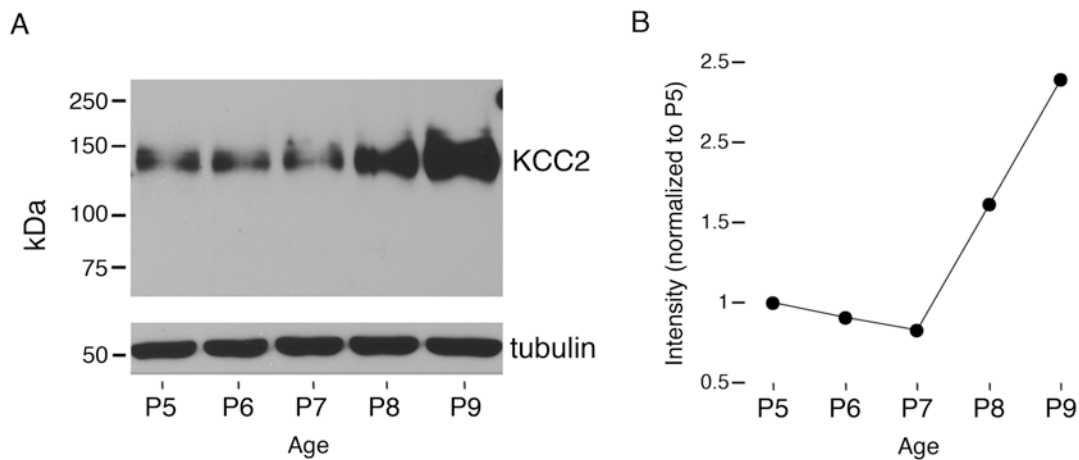
In a subset of subjects that received muscimol infusions (n=3), fluorescently labeled muscimol was infused to confirm the desired distribution of the drug (Figure 8B). Muscimol was concentrated near the site of infusion, although some diffusion to other cortical and subcortical areas occurred. Diffusion to the contralateral hemisphere was negligible. Infusions of bicuculline were not fluorescently labeled as distribution was likely to be similar to that of muscimol.

To assess developmental changes in GABAergic modulation of SBs, we examined in P10 subjects the effects of muscimol and bicuculline infusion. However, in contrast with its effects at P4, infusion of bicuculline at P10 produced a hyperexcitable state, including epileptiform activity. That such activity would occur after but not before P7 in response to GABA antagonism is consistent with a previous report examining age-related differences in epileptiform activity in neocortical slices (Wong & Yamada, 2001). Unfortunately, the nearly continuous cortical activation and epileptiform-like cortical state induced by bicuculline precluded quantification of SB activity.

KCC2 upregulates at the end of the first postnatal week

The inhibitory and excitatory effects of muscimol and bicuculline, respectively, on SB activity at P4 are consistent with GABA exerting its postsynaptic effects through either shunting inhibition or hyperpolarization. Upregulation of the  $K^+/Cl^-$  co-transporter 2, KCC2, through its control of the  $Cl^-$  reversal potential, is thought to be a critical factor in the postnatal emergence of GABA's hyperpolarizing effects (Sipila et al., 2010). Although KCC2 is known to be upregulated sometime during the first two postnatal weeks in the cortex

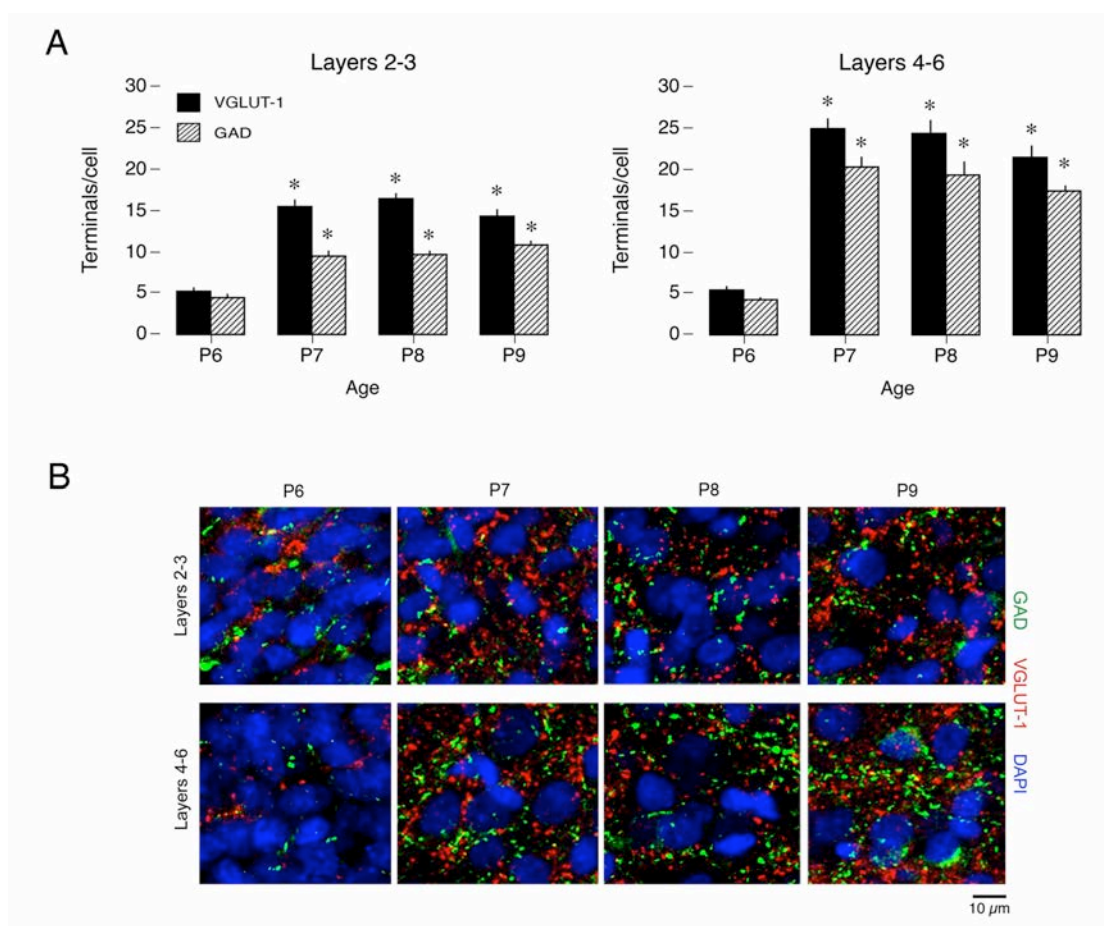
(Wang et al., 2002; Blaesse et al., 2006), protein levels have not been examined on a day-by-day basis. Therefore, we assessed KCC2 levels in cortex from P5, P6, P7, P8, and P9 rats using Western blot analysis. As shown in Figure 9 (left), levels of KCC2 increase substantially at the end of the first postnatal week, with an abrupt increase between P7 and P8.



**Figure 9: Cortical KCC2 protein levels increase at the end of the first postnatal week.** (A) Western blot analysis of KCC2 expression in rat cortex at P5, P6, P7, P8, and P9. kDa, kilodalton. (B) The intensity of KCC2 bands normalized to P5. KCC2 levels are stable between P5 and P7, but increase sharply thereafter.

### Changes in GABAergic and glutamatergic terminals

We examined whether the rapid changes in SB production between P6 and P9 are accompanied by anatomical changes in GABAergic and glutamatergic presynaptic terminals in S1. Specifically, in an additional set of sham subjects (n=3 pups/age; 6 fields/pup), we stained cryosections with antibodies directed against glutamic acid decarboxylase (GAD) and vesicular glutamate transporter-1 (VGLUT1) in layers 2-3 and 4-6 in S1. We controlled for any variation in cell density by normalizing the number of presynaptic terminals to the number of cell nuclei in each field (see Materials and Methods). We found that GABAergic and glutamatergic presynaptic terminals increased rapidly and significantly at P7 in relation to P6, especially in the deep layers (Figure 10A;  $P_s < 0.0001$ ).

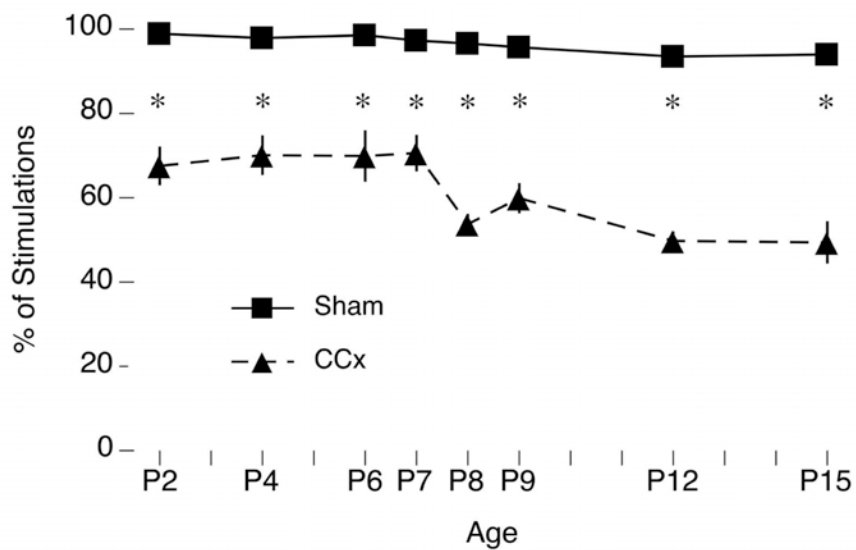


**Figure 10. Changes in GABAergic and glutamatergic presynaptic terminals in P6-9 rats.** (A) Number of S1 glutamatergic (VGLUT-1) and GABAergic (GAD) presynaptic terminals in layers 2-3 (left) and 4-6 (right), normalized to the number of cell nuclei, increase significantly at P7 in relation to P6. \* significant difference from value at P6. Means + sem. (B) Sections through S1 cortex in P6, P7, P8, or P9 sham-operated animals were stained with antibodies against inhibitory (GAD, green) and excitatory (VGLut-1, red) presynaptic terminals; nuclei were counterstained with DAPI (blue). The density of both GABAergic and glutamatergic terminals in layers 2-3 (top row) and layers 4-6 (bottom row) increases sharply between P6 and P7, with little subsequent change over the next two days.

The marked increase in GAD and VGLUT1 terminals between P6 and P7 can be seen clearly in representative immunofluorescent fields from layers 2-3 and 4-6 (Figure 10B). Along with this sharp increase in the number of presynaptic terminals, cortical cells appeared more widely spaced at P7 and, later, resulted in a reduced number of cells per microscope field. This increased spacing is almost certainly due to the rapid expansion of the neuropil and continued growth of the cortex, as no apoptosis was observed between P6 and P7 (based on immunostaining for cleaved caspase-3; data not shown). As noted above, we normalized our synaptic terminal counts to the cell density in each microscope field; thus, these changes in cell density cannot account for the observed increase in the number of presynaptic terminals. Indeed, the absolute (i.e., not normalized) number of presynaptic terminals also exhibited a sharp increase after P6 despite the decrease in cell density. Thus, these histological findings indicate a rapid and steep increase in cortical innervation after P6 that coincides with the loss of the disinhibitory effects of CCx, the upregulation of KCC2, and the developmental decrease in myoclonic twitching.

#### Disruptive effects of CCx on evoked spindle bursts

The effect of CCx on SB responses in the S1 forelimb region to peripheral stimulation to the plantar surface of the contralateral forepaw was measured. In sham subjects, forepaw stimulation reliably evoked an SB response at all ages (Figure 11). In contrast, at all ages tested, CCx significantly reduced the probability of an evoked response by 30-50% ( $P_s < 0.0001$ ).



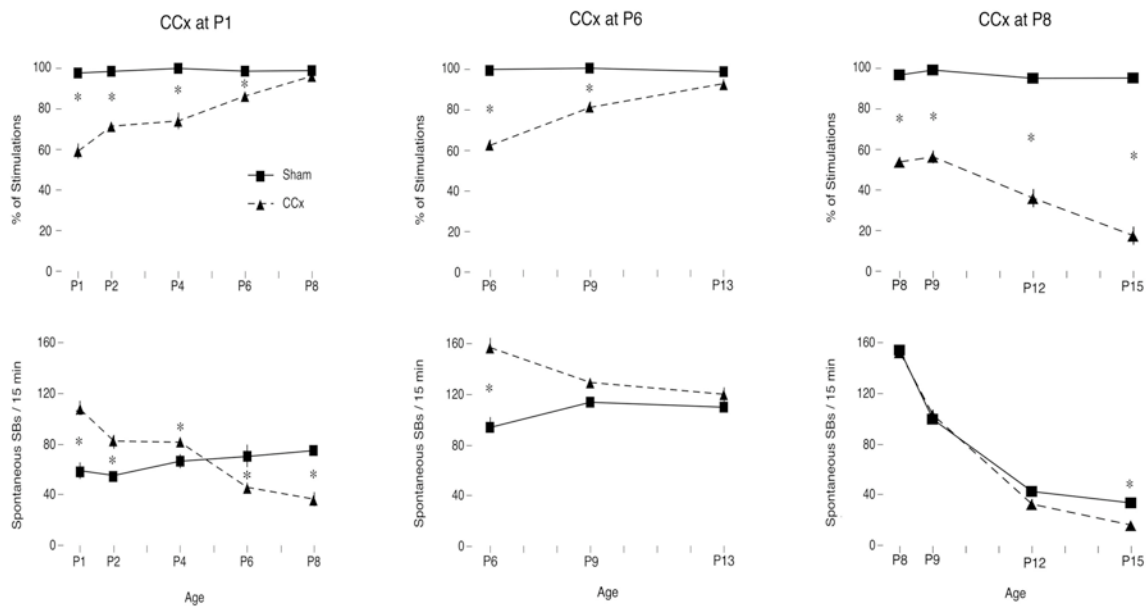
**Figure 11. Callosotomy (CCx) disrupts the SB response to peripheral stimulation of the contralateral forepaw in P2-15 rats.** In sham subjects the response to stimulation of the forepaw reliably evokes an SB in contralateral S1 in P2-P15 infant rats; however, CCx immediately decreases the response to forepaw stimulation at all ages. \* significant difference between groups. Means + sem.



## Development and recovery of function after CCx

Sham or CCx surgeries at P1 or P8 with littermates tested immediately or up to one week later were performed to assess recovery of function across the early postnatal period. As shown in Figure 12, CCx at P1 immediately disrupted evoked responding to forepaw stimulation and also disinhibited spontaneous SBs. However, over the ensuing week, evoked responding gradually recovered to sham levels and spontaneous SBs steadily declined, eventually reaching levels below that of shams. In contrast, CCx at P8 was associated with an immediate disruption of evoked responding followed by steady deterioration of responding thereafter. Interestingly, this disruption of evoked responding after CCx at P8 occurred without any observed effect on spontaneous SB production.

The window for plasticity was examined in greater detail by repeating the experiment at P6 (Figure 12). As with CCx at P1 but in contrast with CCx at P8, CCx at P6 immediately disrupted evoked responding but, over the following week, a steady recovery of function, back up to sham levels, was observed. Additionally, spontaneous SB production was disinhibited in P6 subjects after CCx and returned to sham levels at subsequent ages.



**Figure 12. Disruption of evoked spindle burst responses by callosotomy (CCx) and recovery of function across different ages.** Effects of sham (filled squares) and CCx (filled triangles) surgery on evoked (top) and spontaneous (bottom) cortical activity when surgeries were performed at P1, P6, or P8 with subsequent testing over the ensuing week. \* significant difference between groups. Means + sem.

## Discussion

Here, contemporaneous behavioral, neurophysiological, anatomical, and molecular changes that suggest a role for sleep-related motor activity and its associated sensory feedback in modifying the structure and function of cortical circuits during early development are documented. A rapid decrease in myoclonic twitching beginning at P7 coincided with a decrease in CCx-induced disinhibition of spontaneous SB activity. In addition, both GABAergic and glutamatergic innervation of superficial and deep cortical layers increased sharply at P7 as the rate of spontaneous SBs in both sham and CCx subjects increased dramatically, peaked at P8, and then decreased over the next week. KCC2 expression also increased across these ages, suggesting that developmental changes in GABAergic functioning alter the intrinsic excitability of local cortical circuits involved in the production of SBs. Finally, these behavioral, neurophysiological, neuroanatomical, and molecular changes after P6 were accompanied by an abrupt decrease in somatosensory cortical plasticity.

Myoclonic twitch movements of the limbs, tail, and head are produced by phasic, rapid activation of skeletal muscles (Gramsbergen et al., 1970a; Karlsson & Blumberg, 2002; Seelke et al., 2005a); for review, see (Blumberg & Seelke, 2010). Neurons within the mesopontine region participate in the production of twitches during the early postnatal period (Kreider & Blumberg, 2000; Karlsson & Blumberg, 2005). Recently, it was discovered that sensory feedback from twitching produces SBs in somatosensory cortex (Khazipov et al., 2004); moreover, it appears that it is the proprioceptive feedback from limb twitching that triggers somatosensory SBs (Marcano-Reik & Blumberg, 2008). In addition, SBs are produced in visual (Hanganu et al., 2007) and barrel (Minlebaev et al.,

2007) cortex, and hippocampal activity is also modulated by sensory feedback from twitching (Mohns & Blumberg, 2008; 2010). Mohns and Blumberg have further demonstrated that myoclonic twitches are consistently followed by SBs, which are in turn followed by bursts in hippocampal unit activity. Thus, it is now clear that sensory feedback from twitching modulates neural activity throughout the neuraxis – from spinal cord (Petersson et al., 2003) to forebrain – thus supporting the hypothesis that sleep-related endogenous activity shapes and refines neural circuits in early development and across the lifespan (Roffwarg et al., 1966; Corner & Kwee, 1976; Corner, 1977; Blumberg & Lucas, 1996; Blumberg & Seelke, 2010).

From the data in Figure 7 – and given that circadian effects on sleep-wake activity at these ages are relatively small (Gall et al., 2008) – we can estimate that the nuchal muscle (at least in the region from which we recorded) twitches over 38,000 times per 24 hours at P6; this number decreases to 9,500 at P9, a four-fold decrease over 3 days. Within the forelimb region of somatosensory cortex, we can estimate that nearly 4,000 SBs occur per 24 hours at P6, a value that jumps to over 15,000 at P8 before declining to levels below 4,000 by P12. Although these values are only estimates, they serve to highlight the considerable quantity of endogenous activity produced and experienced by infant rats over the first two postnatal weeks. Thus, in early development, twitching may serve a similar role for the somatosensory cortex as retinal waves serve for the visual cortex (Katz & Shatz, 1996; Wong, 1999). It is not yet clear whether the effects of twitching on the development of somatosensory cortex are likely the result of permissive or instructive influences (Crair, 1999).

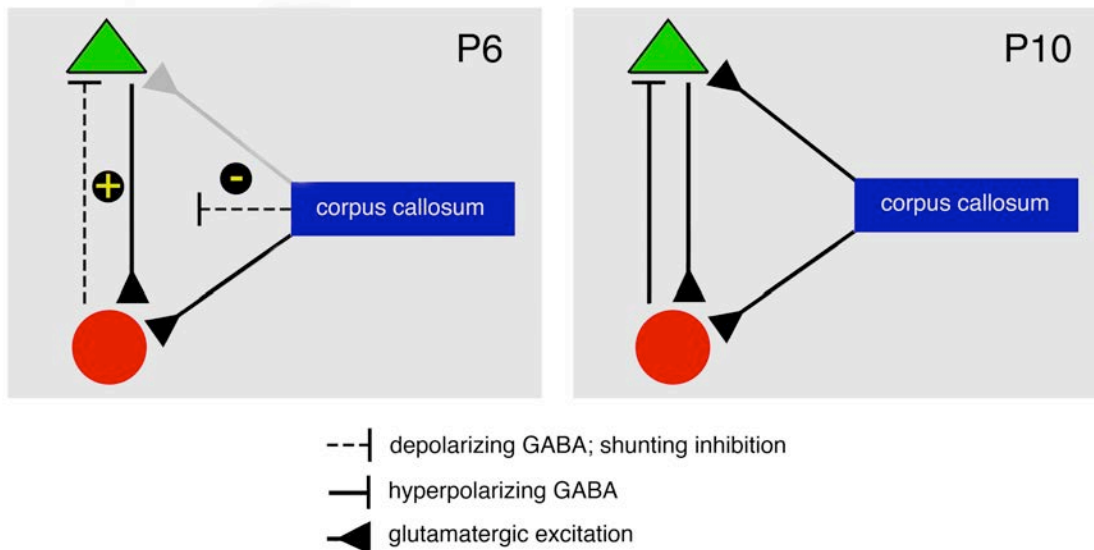
Our findings suggest that callosal fibers inhibit spontaneous SBs during the first postnatal week via excitatory effects on GABAergic interneurons. Although activation of GABA<sub>A</sub> receptors typically depolarizes postsynaptic cells in immature cortex before the developmental upregulation of KCC2, GABA-mediated inhibition can still occur as a result of shunting inhibition of glutamatergic excitatory postsynaptic currents (Lamsa et al., 2000; Ben-Ari, 2002; Owens & Kriegstein, 2002; Akerman & Cline, 2007; Sipila et al., 2010). For example, similar to what was found here, in the newborn hippocampus, muscimol and bicuculline decrease and increase network activity, respectively (Lamsa et al., 2000). However, the pharmacological manipulations used here are not sufficient to discern the mechanism by which GABA inhibits postsynaptic activity.

The fact that KCC2 is upregulated in the cortex at the end of the first postnatal week suggests that GABA does indeed exert depolarizing effects at earlier ages. This upregulation is also consistent with previous findings in cortical tissue across a broader range of ages. Specifically, KCC2 mRNA in cortex increases between P1 and P15 (Wang et al., 2002) and KCC2 immunoreactivity is very low at P4 and uniformly high at P12 (Blaesse et al., 2006). In rat neocortical brain slices, the GABA<sub>A</sub> receptor reversal potential approaches the resting membrane potential sometime after P4 (Owens et al., 1996). Similar to what we found here in cortex, KCC2 expression in hippocampus also increases sharply between P5 and P9 (Rivera et al., 2005).

The callosal inhibition observed here through P6 may be attributable in part to a transient GABAergic population of callosal neurons comprising approximately 21% of the callosal bundle in infant rats (Cobas et al., 1988;

Kimura & Baughman, 1997). Significantly, these GABAergic neurons largely disappear at or before P6 and few such fibers are detected in adults (Fabri & Manzoni, 2004).

Figure 13 presents a model depicting hypothesized changes in intrinsic cortical circuitry and callosal influences on that circuitry between P6 and P10. At P6, the local circuit producing SBs comprises glutamatergic pyramidal cells and GABAergic interneurons; at this age, GABAergic inhibition occurs through shunting. The fact that CCx disinhibits SB activity at this age suggests net excitation in the intrinsic cortical circuit that is counteracted by a net inhibitory influence provided by callosal excitation of GABAergic interneurons and/or direct inhibition provided by the transient population of GABAergic callosal neurons. By P10, after KCC2 upregulation, the emergence of hyperpolarizing GABA, and the other changes in intrinsic connectivity described here, we hypothesize that excitatory-inhibitory balance is now expressed within the intrinsic cortical circuit. In addition, we hypothesize that callosal fibers, which are thought to exert both excitatory and inhibitory effects in adults (Bloom & Hynd, 2005; Makarov et al., 2008), exert a more balanced influence on the intrinsic circuit by P10. Thus, we view excitatory-inhibitory balance as arising from complex interactions among intrinsic cortical circuits and extrinsic influences that include the corpus callosum.



**Figure 13. Model depicting hypothesized mechanisms underlying the development of excitatory-inhibitory balance in S1 between P6 and P10.** The model depicts an S1 network comprising GABAergic interneurons (red circles) and glutamatergic pyramidal cells (green triangles) combined with callosal modulation of the intrinsic circuit. Left: At P6, CCx disinhibits SB activity in S1, suggesting net excitation (+) in the intrinsic cortical circuit and a net inhibitory influence (-) by the corpus callosum. Although GABA is typically depolarizing early in development, GABA-mediated inhibition can still occur as a result of shunting of glutamatergic excitatory postsynaptic currents (dashed line). According to this model, net callosal inhibition is achieved by preferential excitation of GABAergic interneurons as well as the presence of transient GABAergic callosal projections (most likely by inhibiting pyramidal cells, although such connectivity is not specified in the figure). Right: At P10 and after KCC2 upregulation, the hyperpolarizing effects of GABA predominate and the intrinsic cortical circuit has achieved excitatory-inhibitory balance. In addition, callosal inputs from the corpus callosum now exert equal effects on excitatory and inhibitory cortical neurons and GABAergic callosal projections have disappeared. As a consequence, CCx at this age has no discernible effect on spontaneous activity (although evoked responses are still affected).

In addition to their neurophysiological effects, glutamate and GABA may act as trophic signals in early development that, among other things, stimulate the release of brain-derived neurotrophic factor (BDNF) (Marty et al., 1996; Ben-Ari, 2002; Owens & Kriegstein, 2002; Xiong et al., 2002; Marmigere et al., 2003; Akerman & Cline, 2007). BDNF is produced by cortical pyramidal cells and, in an activity-dependent manner, regulates synaptic interactions among glutamatergic pyramidal cells and GABAergic interneurons (Turrigiano, 1999). As a consequence, twitch-triggered SBs in the developing neocortex may, through the release of glutamate, GABA and BDNF, contribute to the somatosensory cortical plasticity observed here during the first postnatal week. Moreover, the endogenously generated SBs may provide the activity upon which KCC2 upregulation and the emergence of hyperpolarizing GABA depend. This activity may be mediated by GABA itself (Ganguly et al., 2001), by glutamate (Kanold, 2009), or by other factors (Ludwig et al., 2003).

After P6, as rates of twitching sharply decline and GABA no longer exerts depolarizing effects, we hypothesize that associated declines in the activity-dependent release of BDNF will lead to relative increases in excitatory drive onto pyramidal cells, as has been hypothesized for cultured cortical networks (Turrigiano, 1999). These adjustments in network organization, which may also include the observed relative increases in glutamatergic presynaptic terminals, may contribute to the peak in SB activity at P8 and the emergence of excitatory-inhibitory balance (Owens & Kriegstein, 2002; Hensch, 2005; Akerman & Cline, 2007).

In light of all of these foregoing issues, we predicted that CCx-induced disinhibition during the first postnatal week would serve as a bioassay of



somatosensory cortical plasticity. To test this prediction, we performed sham or CCx surgeries at P1, P6, or P8 and tested littermates immediately or over the ensuing week. Although the evoked response to contralateral forepaw stimulation is typically very reliable, CCx consistently disrupted evoked responses to forepaw stimulation. Only pups receiving CCx at P1 or P6 – ages at which CCx disinhibits spontaneous SB activity – exhibited recovery of function. The evoked SB responses of pups that received CCx at P8 deteriorated further over the week. In addition, as evoked responses recovered, inhibitory control of spontaneous responses appeared to reemerge (see Figure 12). Although we do not yet know whether CCx-induced disinhibition of spontaneous SBs is causally related to processes underlying recovery of function in S1, it does appear that disinhibition can be used as a bioassay of conditions conducive to recovery of function. Moreover, the present findings suggest a new model for exploring somatosensory cortical plasticity.

It is interesting that CCx before P6 doubles the expression of spontaneous SBs but decreases the likelihood of evoked SBs to contralateral forepaw stimulation (compare the developmental profiles in Figures 6A and 9). We have previously reported other pronounced differences between spontaneous and evoked SBs (Marcano-Reik & Blumberg, 2008). Such differences could reflect the contributions of non-overlapping neural circuits to the production of spontaneous and evoked SBs, including the production of an efference copy in association with spontaneous SBs. Efference copy provides a mechanism by which animals distinguish sensations arising from self-generated or passive movements (Blakemore et al., 2000; Cullen, 2004). Thus, we hypothesize that efference copy associated with self-generated twitches, perhaps communicated

through the basal forebrain (Rasmusson & Dykes, 1988; Ma et al., 1989; Tremblay et al., 1990; Juliano et al., 1991; Kilgard & Merzenich, 1998), prepares the neocortex for twitch-related sensory feedback, thereby contributing to sensorimotor development and somatotopic organization. Interestingly, cholinergic afferents from the basal forebrain facilitate early spindle burst activity in primary visual cortex (V1) during a critical period in early postnatal development (Hanganu et al., 2007).

All together, the changes in twitch behavior and associated SB activity observed here suggest that the first postnatal week in rats ends with an increase in intrinsic cortical synaptic connectivity, a transformation in GABA's functional properties, the establishment of excitatory-inhibitory balance, and a loss of somatosensory cortical plasticity. These findings also suggest that the corpus callosum participates in the activity-dependent development and refinement of cortical networks and point the way to an understanding of how malformations of the corpus callosum may contribute to a variety of neurological and psychiatric disorders (Paul et al., 2007).

## CHAPTER IV GENERAL DISCUSSION

If, as suggested, sleep-related twitches and their associated sensory feedback (i.e., SBs) contribute to the refinement of somatosensory cortical development soon after birth (Khazipov et al., 2004), then it is possible that twitches and SBs also contribute to plasticity and recovery of function in S1. As described earlier, the corpus callosum modulates SBs during early development and CCx disinhibits spontaneous sleep-related SBs through, but not after, postnatal day 6 (P6) in infant rats. This disinhibition is a transient feature of early cortical development and also correlates with a period of heightened somatosensory cortical plasticity. When CCx-induced disinhibition of SBs disappears, somatosensory cortical plasticity and the mechanisms and factors that support recovery of function also disappear.

These findings document several developmental changes that occur after CCx-induced disinhibition disappears including a decrease in active sleep-related myoclonic twitch rates, a peak in spontaneous SB activity in S1 in both sham and CCx subjects, a dramatic increase in both GABAergic and glutamatergic presynaptic terminals in superficial and deep layers in S1, an upregulation in KCC2 expression in S1, and the closing of a critical period of somatosensory cortical plasticity (Marcano-Reik et al., 2010). Although we have identified potential factors that contribute to somatosensory cortical plasticity during early development, including GABA and KCC2, the role of these rapidly changing mechanisms and how they interact in facilitating or preventing somatosensory cortical plasticity and recovery of function is not yet clear.

These findings support a transient period of S1 cortical plasticity that is modulated by the corpus callosum and directly influenced by sensory feedback from active-sleep-related myoclonic twitches. These findings may provide insight into biological and functional disruptions that underlie various neurologic and psychiatric conditions. Recently, Dinstein and colleagues reported that interhemispheric synchronization of cortical activity was disrupted during sleep in toddlers with autism (Dinstein, 2011). Therefore, it is possible that asynchronous interhemispheric cortical activity may serve as a pathophysiological measure of early autistic symptoms.

Callosotomy, and the resultant hyperexcitable cortical state in early development, offer a new and unique model system for examining the effects of early perturbations of cortical circuitry on somatosensory development, cortical plasticity, and recovery of function after injury. Sleep-related myoclonic twitches, which occur predominantly during the perinatal period, may serve as a primary source of sensory input driving activity-dependent processes in somatosensory cortical development, similar to the role played by retinal waves in the development of the visual cortex (Katz & Shatz, 1996; Wong, 1999). Future examination and comparison of these two developing sensory systems should take into account the effects of altered peripheral inputs (i.e., limb paralysis or dorsal rhizotomy vs. monocular deprivation or ocular transection) on primary sensory cortical development and sensorimotor organization.

A potential limitation of the experiments presented throughout this dissertation involves the inability to include video recording of myoclonic twitch behavior during test sessions. Although behavioral observation of myoclonic twitches was observed and marked in synchrony by the observer with the

electrophysiologic record during the session, a video record would have allowed for synchronization of a digital file of behavioral activity with the EEG and EMG records. In addition, video records of sleep- and wake-related behaviors would have allowed for slow-motion playback for detailed examination of distal and proximal myoclonic twitches and their contribution to somatosensory cortical development and plasticity. This may have also allowed for a temporal analysis of these primary developmental factors during the early postnatal period: behavior, cortical activity, and muscle twitch activity.

Future examinations of sleep-related motor behavior and the associated sensory cortical activity should take into consideration the rapid changes that occur over a very short period of early development. Our results indicate that recovery of function (as measured by the evoked SB response in S1 to peripheral stimulation of the forepaw) is only evident through, but not after, the first postnatal week of development in infant rats. Therefore, an examination of neurochemical concentrations and properties *in vivo* – such as brain-derived neurotrophic factor (BDNF), GABA, and glutamate – that change during this transient period, would provide further insight into mechanisms that contribute to structural and functional organization of the injured nervous system.

The above research findings and model describe the role of sleep-related motor activity (i.e., myoclonic twitches) and associated sensory feedback (i.e., spindle bursts; SBs) on early developing somatosensory cortical networks. Although the contribution of twitches to the self-organization of the nervous system has been described before (Petersson et al., 2003; Khazipov et al., 2004; Petersson et al., 2004), the role of sleep-related twitches and their associated sensory feedback in early cortical development and recovery of function after

cortical damage has not been addressed. Therefore, it seems reasonable to suggest that future models of injury to the developing nervous system take into account sleep-related sensorimotor activity, which is callosally mediated, and its influence on mechanisms that contribute to a transient period of plasticity during early postnatal development.

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